Optimization and Validation of a Pathogenesis Survival Assay with the *C. elegans* Sterile Mutant *Fer-1*

A Major Qualifying Project submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science

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Abstract

An infertile *C. elegans* mutant *fer-1* was identified as a suitable substitute for wild type in evaluating the pathogenesis of *C. albicans* using a survival assay. The response of *fer-1* mutant worms infected with avirulent *C. albicans* mutants or treated with fluconazole is statistically indistinguishable from the wildtype counterpart. The lifespan of uninfected *fer-1* worms is marginally yet reproducibly shorter than wildtype, but the lack of progeny in sterile mutants makes them an ideal choice for otherwise labor-intensive lifespan analyses.

Acknowledgements

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Introduction

The *Candida albicans* pathogen

Candida albicans' notoriety

Candida albicans is a commensal organism that colonizes humans' mouths, gastrointestinal and uro-genital tracts and exists without harm to the host (Joualt, 2009). However, *C. albicans* colonization is a threatening condition in immunocompromised patients, such as those with HIV/AIDS. Other high risk populations include those under broad-spectrum antibiotic treatment and organ failure (Blumberg, 2001). When the innate immune system of an individual is compromised, his or her ability to fight off a fungal infection is drastically impaired, leading to a high mortality rate (Jarvis, 1995). This risk is particularly high upon infection of the bloodstream – candidemia – primarily seen in patients with intravenous catheters or other medical implants (Blumberg, 2001).

Candida infections are the largest component of nosocomial infections, especially those of the urinary tract, or UTIs (Achkar, 2010). A case study of *Candida* infections reported in the ICU of a particular hospital (Leleu, 2002) disclosed overwhelming statistics. Analyzing candidiasis and candidemia cases at this hospital over a three year period recorded that 0.3% of ICU admissions developed confirmed *Candida* infections, 84% of which are caused by *Candida albicans*. This average can range significantly between hospitals and even reach as high as 1.58% of admissions (Rangel-Frausto, 1999). This study also correlated candidiasis and candidemia with increased mortality, longer hospital stays, acute respiratory distress, shock, and workload on the hospital. Therefore, *Candida* infections are not only a threat to public health, but a stress to the infrastructure and management of the health care system, pressuring their time,

energy, and money. Thus, there is an urgency to learn more about how this specific fungus causes a disease and how the immune system reacts to the infection.

How *C. albicans* infects

Much research has been conducted to investigate the pathways involved in *Candida albicans* virulence. Various studies (Gow, 2002; Money, 2001; Hube, 2001) reveal that the morphological transition from yeast to hyphae (or pseudohyphae) are associated with infection because these morphologies are more invasive. Hyphae have pathogenic advantages that yeast form cells do not have in that they can extend the tips of the hyphae with a considerable amount of pressure, comparable to that of 8% agar (Money, 2001). The hyphae thus poke through and kill the macrophages that ingest the cells (Gow, 2002). Hyphae can also navigate the topography of nearby host cells with the hyphal tips, locating weak, vulnerable spots best for infection – a process called thigmotropism, or contact guidance (Hardham, 2001). In addition, hyphae tips secrete proteases and enzymes that degrade lipids in order to dissolve a path of infection ahead of the cell. Of high interest recently are the secreted aspartyl proteases (SAPs) known to enhance *C. albicans* virulence (Hube, 2001).

These hyphal filamentation processes are controlled by various pathways, including the Czf1, Cph1, Cph2, RascAMP and MAPK pathways and Efg1 transcription factor. Inactivating Tup1 and CaNrg1 as well as activating a pH pathway at an ambient pH cause *Candida* to filament (Gow, 2002). Interestingly, Tup1, Nrg1, Efg1, Cph1 and Cph2 also regulate other virulence phenotypes such as adhesion and the secretion of proteases (Murad, 2001). Despite the advantages of the hyphal form, yeast form cells have been demonstrated to disseminate better than hyphae through the circulatory system. (Gow, 2002). The studies have only scratched the

surface of uncovering the pathogenicity of *C. albicans* and have mainly only applied candidate gene approaches to the research.

Shortage of Anti-fungal Drugs

Currently, drugs available to treat fungal infections are minimal. Antibiotics are very common partially because bacteria are prokaryotic, and have different features such as peptidoglycan in their membranes that allow drugs to work without adversely affecting the human. As fungi are eukaryotic, they share many similarities with their hosts, whether it is an animal or a human. This largely limits the drugs available for patients as many will harm the patient while attacking the fungus. Nevertheless, several antifungal agents have been developed since in the past sixty years, as seen in Figure 1 below.

Class	Generic name	Brand name	Available formulation(s)	Year initially approved
Polyene	Amphotericin B	Fungizone (Apothecon Products, Princeton, NJ)	Intravenous, oral solution	1958
Polyene	Amphotericin B lipid complex	Abelcet (Liposome, Princeton, NJ)	Intravenous	1995
Polyene	Amphotericin B cholesteryl sulfate	Amphotec (SEQUUS Pharmaceuticals, Menlo Park, CA)	Intravenous	1996
Polyene	Amphotericin B liposomal	AmBisome (Fujisawa Healthcare, Deerfield, IL)	Intravenous	1997
Pyrimidine	Flucytosine	Ancoban (ICN Pharmaceuticals, Costa Mesa, CA)	Oral tablet	1972
Azole	Ketoconazole	Nizoral (Janssen Pharmaceutica, Titusville, NJ)	Oral tablet	1981
Azole	Fluconazole	Diflucan (Pfizer, New York, NY)	Intravenous, oral tablet, oral suspension	1990
Azole	Itraconazole	Sporanox (Janssen Pharmaceutica, Titusville, NJ)	Intravenous, oral capsule, oral solution	1992

Figure 1: Known antifungal agents used to treat systemic infections in the United States (Dismukes, 2000)

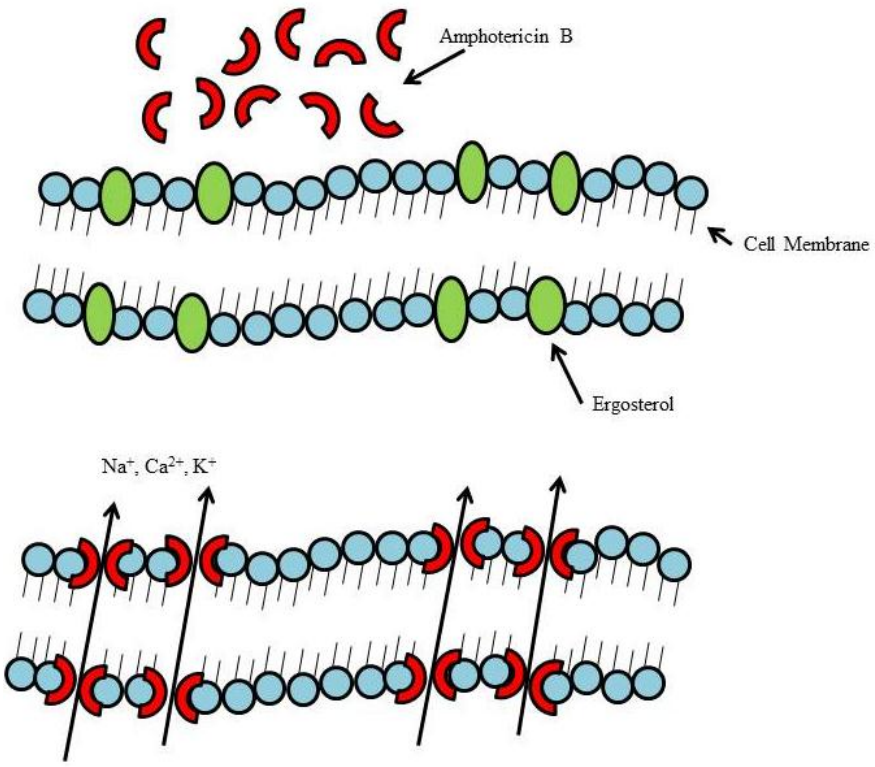

Figure 2: Mechanism of Amphotericin B on a fungus

As an example, the "gold standard" of antifungal drugs is Amphotericin B. It functions by binding with ergosterol and forming channels in the membrane which causes necessary ions such as potassium and sodium to leak out, eventually leading to cell death as shown in Figure 2. However, mammalian cells are composed of cholesterol, a very similar compound. Because they are so similar, Amphotericin B is toxic to the mammal as well as the fungus. Amphotericin B is often a last resort for doctors as it is limited by infusion-related toxicity (Thompson et. al., 2008). However, in the case of a bloodstream infection, Amphotericin B is often the first drug to be used (Anaissie, 1996).

Another family of antifungals that is more commonly used, is Echinocandins, i.e. caspofungin™ (Merck Inc.), are commonly used as a first line of therapy. The side effects aren't frequent, and it has a favorable drug interaction profile (Thompson et. al., 2008). The drug works

by inhibiting the synthesis of β(1,3)-D-glucan, an integral component in fungal cell walls. It inhibits further growth of fungi by eliminating this crucial structural protein. It is also fungicidal in that it removes the stability of the cell, causing it to eventually lyse from osmotic pressure. It is not present in mammalian cells, drastically reducing the issue of toxicity (Letscher-Bru, et al., 2003). However, multiple strains of Candida have become resistant to the drug with prolonged exposure. Specifically with Caspofungin, many of the isolated mutations were linked to the glucan synthase complex which was reported to be a mutation hotspot. In *C. glabrata*, the most common mutation seen was F659V, and in *C. krusei*, R1361G (Thompson et. al., 2008).

Another common, first line antifungal drug family is the azole family. There are many different azoles, including fluconazole, itraconazole, and voriconazole. Fluconazole is one of the more well-known and prescribed antifungal medications. There have been numerous studies on fluconazole as well as Candida's ability to resist the drug.

Azole drugs target the enzyme lanosterol demethylase in the ergosterol biosynthetic pathway (Figure 3). Instead of incorporating ergosterol into the membrane, toxic sterols are instead included which compromise the stability of the membrane. Perea *et al.* found that fluconazole resistance did not infer resistance to itraconazole and other members of the –azole family. However, many patients had a decreased susceptibility to the other drugs in the family. It was seen that the most common mechanism for resistance was efflux pumps. Fluconazole resistant strains were not resistant to Amphotericin B as the mechanisms are not comparable and the drugs are not related. Therefore resistance to one would not cause resistance to the other (Perea et al. 2001).

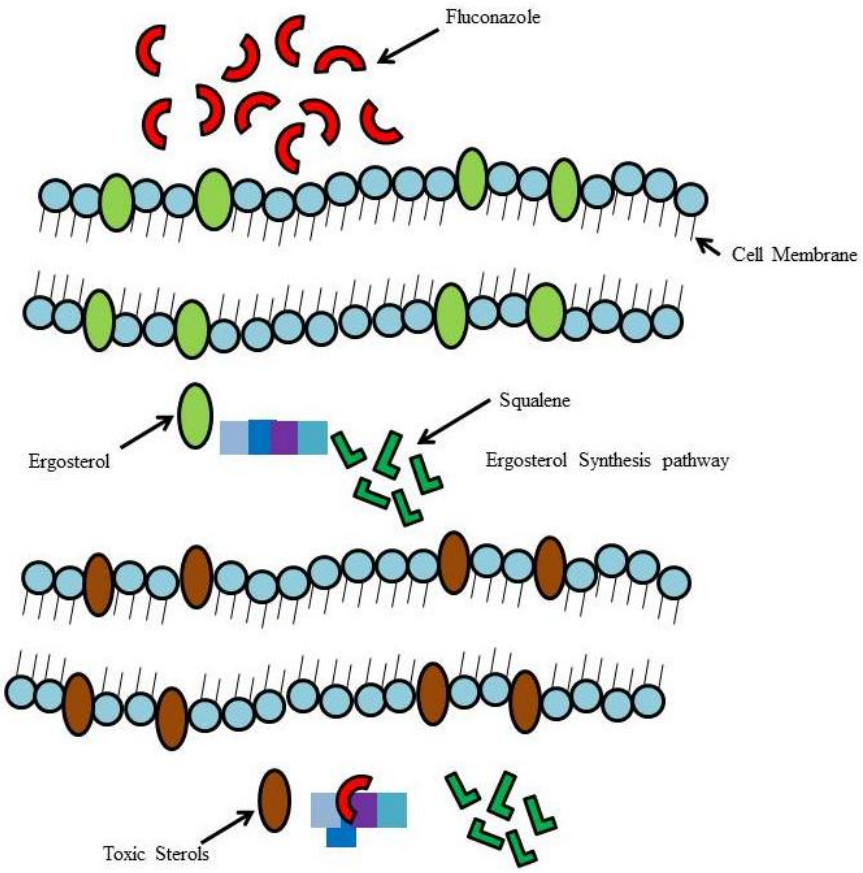

Figure 3: Mechanism by which an azole works on a fungus

It was found that many of the genes that control efflux pumps were either upregulated or overexpressed in almost all of the patients studied. Specifically, CDR genes were overexpressed: *MDR1* and *ERG1* were upregulated in most of the patients (Perea et al. 2001). This increase in efflux pumps prevents azoles from reaching a high enough concentration to destabilize the membrane through the production of toxic sterols in the membrane.

When the strains that were fluconazole resistant were isolated and analyzed, eleven different mutations were found. Nine of the mutations had previously been isolated. Because many of these mutations occur near each other with respect to base pair number, it indicates that there are mutational hot spots. In this case, it was found that many of the mutations occurred in regions that involved binding or the active site (Perea, et al. 2001).

There are many strains that are resistant to different antifungal drugs. The development of chemosensitizers, or other compounds that can surpass resistance is part of a new method for improving antifungal drugs. Youngsaye *et al.* (2011) found that piperazinyl quinolone functioned as a chemosensitizer, increasing the susceptibility of *Candida* to fluconazole. Even though many compounds were tested, piperazinyl quinolone was found to be the most effective chemosensitizer. This compound's use as a chemosensitizer is not necessarily feasible as it is not easily soluble. Benefits of the compound are that alone, it is not inherently toxic or fungicidal. Also, it did not act through an established resistance pathway.

Oloki *et al*. (2009) screened a library of 3,228 compounds, and isolated nineteen of which have antifungal abilities. Seven of the isolated compounds are known antifungal drugs. The other twelve compounds fall into three different categories: the compound is not soluble in water, the compound kills the worms as well as the fungus, and the compound is not effective against the specific fungus used in the assay but is effective against most others. Most of the isolated compounds have antifungal activity, but have problems that would derail its clinical use. For example, concanamycin A had the highest antifungal activity of the compounds tested, but it is highly toxic. Some of the other compounds isolated appeared to have antifungal effects *in vivo* but not *in vitro*.

In collaboration with the Kaufman lab, the Prusty Rao lab completed a screen for possible compounds that could be used as a component to an antifungal. Out of the 30,000 compounds screened, one was isolated for further testing in mice after utilizing the *C. elegans* assay as a secondary screen.

C. elegans for an innate immune system model

Model organisms for the host immune system

Various animals, including the fruit fly *Drosophila melanogaster*, the caterpillar *Galleria mellonella* and mice, are studied for their immune systems' reactions to various pathogens. To infect the animal, it is injected with the pathogenic bacteria or fungus to detect changes and components that may be related to the innate immune system (Miyata, 2003). Specific responses in *Drosophila* to the fungus *B. bassiana* and the Gram-negative bacteria *S. marcescens* and *E. carotovora* have been studied, among other pathogens, revealing a Toll-like receptor pathway and JNK pathway similar to that of humans, and an induced antimicrobial peptide entitled drosomycin (Leclerc, 2004). The innate immune responses in *Galleria* to various fungi (Mylonakis, 2008) and the bacteria *P. aeruginosa* (Miyata, 2003) have been elucidated and a myriad of immunological investigations in mice have been conducted as well.

An animal that has gained popularity for studying host innate immune responses is *C. elegans*. Although the nematode does not have an acquired immune system like humans, its innate immune system – the process by which fungal infections are predominantly fought – has many analogous features and processes. Moreover, the worm *C. elegans* is an ideal model for studying host immune system responses because of its short lifespan of about three weeks, the simplicity of infecting the worm by plainly feeding it the pathogen, and the ability of the hermaphrodite to self-fertilize, which together allow for relatively straightforward, high-throughput genetic screens and survival assays (Kurz, 2000).

In genetic analyses of *C. elegans*, mutagenesis is done on a first generation, and their progeny, F2, are observed for certain phenotypes. Since the worms self-fertilize, a mutation should be homozygous and thus visibly present in a quarter of the F2 progeny. Another

advantageous characteristic of *C. elegans* for use in genetic analyses is its arrested growth in the absence of food, known as the dauer stage. Worms slow their metabolism and remain arrested in growth, which may be used to synchronize them for use in a survival or an infection assay. Finally, *C. elegans* is an excellent model for genetic study because compared to screens with mice and other mammalian systems, the worms take a great deal less time, labor and money and because facets of the innate immune response, the main defense against fungal infections, is conserved between humans and *C. elegans* (Kurz, 2003). Throughout the course of a survival assay, there are various levels of *C. albicans* pathogenesis and of disease in the worms. Below is a depiction (Figure 4) of an infected *C. elegans* at its worst, when hyphae take over the organism and burst through it.

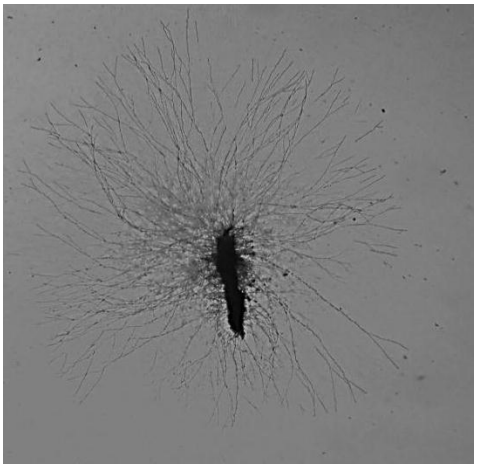

Figure 4: *C. elegans* infected with *C. albicans*
The *C. albicans* that the *C. elegans* ingested converted to the pathogenic hyphal form, poking through the cuticle of the worm and killing it.

The *C. elegans* innate immune system

C. elegans is an ideal organism for conducting genetic analyses and observing immune responses because its antimicrobial peptides, MAPK pathway and several other processes are comparable to those of the human innate immune system. The first level of the *C. elegans* immune system that is analogous to humans' entails the secretion of antimicrobial peptides, or

AMPs from epithelial cells in the mouth and gastrointestinal tract. Additionally, the grinder made of chitin in the pharynx breaks down most pathogens that are ingested, since *C. elegans* feeds mainly on microorganisms (Kurz 2003). The AMP molecules are rich in glycine and tyrosine (Pujol, 2008), are small (about thirty to forty residues long) and their genes are often assembled as operons. One AMP, abf-2, was detected in excretory cells with a GFP tag and discovered to be somewhat effective against several fungal species: *S. cerevisiae, P. anomala, D. hansenii, C. krusei* (Kato, 2002). Antimicrobial peptides are fascinating to study because they evolve very quickly and frequently, as a result of the "battle" between host and pathogen – co-evolution (Netea, 2008).

After the initial level of defense at the epithelial cells, a series of cellular processes for microbes in general or specifically for fungal pathogens are activated in response to infection. Well known in cellular biology is the p38 mitogen-activated protein kinase pathway, or MAPK pathway, which partakes in immune system responses against pathogens. While sek-1 and nsy-1 are proteins identified in the human MAPK pathway, esp-2 and esp-8 were identified as the homologous proteins in the *C. elegans* MAPK pathway (Kim, 2002). These proteins, sek-1 and nsy-1, activate pmk-1, which in turn stimulates pathogen-fighting cells like macrophages and interleukins (Kurz, 2003). The proteins of the MAPK pathway also promote apoptosis in cells already affected and damaged by the infection (Aballay, 2001). Downstream of these proteins in the MAPK pathway in *C. elegans* are nlp29 and 31, neuropeptide-like proteins that are activated by NIPI3, the protein analogous to Tribbles homolog 1 in humans based on their catalytic domains (Pujol, 2008). Furthermore, the extracellular-signal-regulated protein kinase (ERK), the JUN kinase (Kurz, 2003) and the TGFβ signaling pathways (Tan, 2001) contribute to the activation of immune responses.

Host responses specific to *C. albicans*

The above pathways are general immune system responses to most pathogens, but there are some responses specific to fungi. On the surface of the cell are PAMPs (pathogen-associated molecular patterns) called glycophosphatidylinositol (GPI)-anchor-dependent cell wall proteins unique to fungi, making them identifiable to the immune system. These proteins are highly glycosylated with polysaccharides containing mannose, known as mannan. The two main types of mannan are O-linked mannan which is short and linear and N-linked mannan which is highly branched (Netea, 2008). In fact, N-linked mannan has been directly related to candidiasis and fungal load in mice organs. Mannose receptors (MR) on the surface of macrophages and monocytes recognize and bind the N-linked mannosyl residues and Toll-like receptor (TLR) 4 recognizes O-linked mannosyl residues (See Figure 4 below). Recognition at these receptors, especially MR, stimulates cytokines like TNFα, IL-1β, IFN-γ and IL-6. Some cytokine stimulation is brought about with the help of MyD88, the myeloid differentiation primary response gene 88, which is an intracellular TLR adaptor molecule. Minimal cytokine stimulation (IL-2, -10 and -17) results from laminarin, a *C. albicans* ligand rich in β1,3-glucan (Netea, 2006). The β-glucans are detected by the dectin-1 receptor, but can only be bound, along with chitin, when exposed because a new cell buds. β-glucans and chitin are at the core of the cell wall, and they hydrogen-bond to create a strong network of fibers (Netea, 2008). Stimulating these various cytokines in turn recruits neutrophils, macrophages and monocytes to ingest the *Candida* (Van't Wout, 1988) and polymorphonuclear (PMN) leukocytes (Netea, 2008).

Besides inducing cytokines and ingesting pathogenic cells, toxic oxygen and nitrogen radicals are a strong defense mechanism used by the host to fight infection (Djeu, 1990). In a

process known as respiratory burst, toxic and oxidative free radicals activate proteases that attack the invading microorganism (Aratani, 1999).

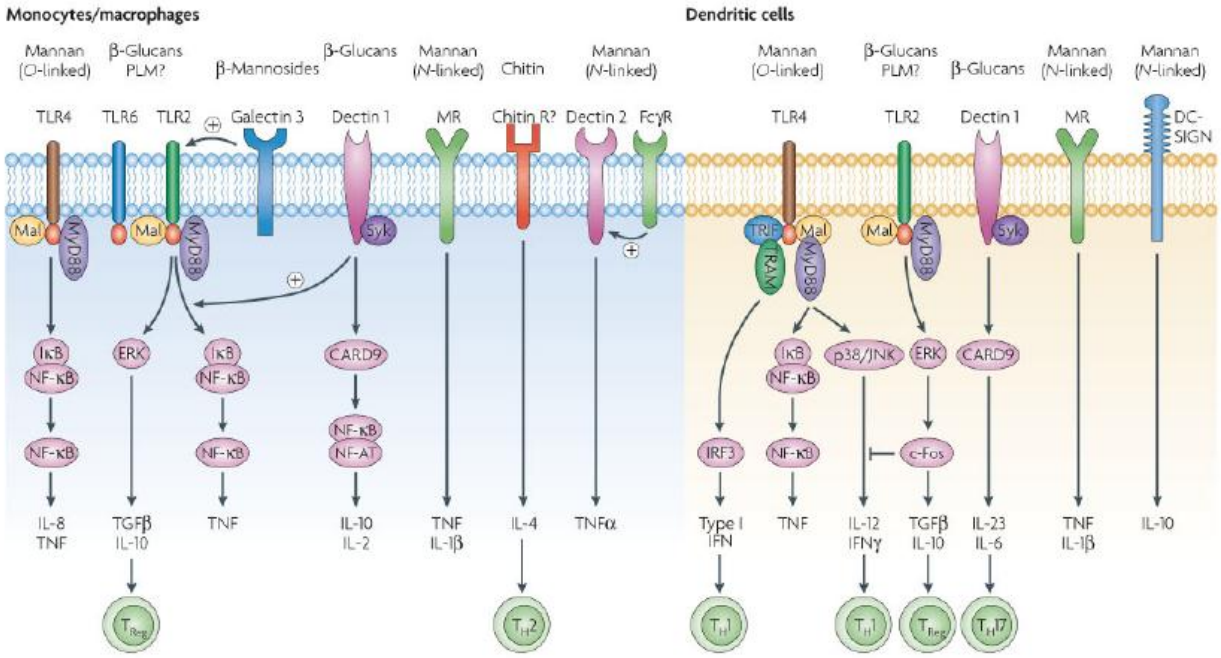

Figure 5: Receptors, PAMPs and consequent pathways activated in response to Candida albicans infection (Netea, 2008)

In addition to the pro-inflammatory responses described, *Candida albicans* also causes anti-inflammatory responses, because of its status as a commensal organism and because of its dimorphic nature. For instance, dectin 2 specifically recognizes hyphae, especially its mannose. Moreover, the receptor CR3 that recognizes β-glucans is immunosuppressive (Netea, 2008), as are the cytokines IL-4 and IL-10 (Tonnetti, 1995). There is a fine line – a balance – between controlling the commensal organism or low-grade infection, and a progression into disease (Romani, 2004). For this reason, there are various pathways with different magnitudes and kinetics to become specific to yeast or hyphae, commensal organism or pathogen (Netea, 2008).

The pathogenesis assay

C. elegans was chosen for this research over mice, fruit flies and caterpillars for a multitude of reasons. The worms' innate immune system is similar to that of humans, which is the part of the immune system that fights off fungal infections. It is also capable of simply ingesting the *Candida* off the agar plate where *C. elegans* lives, whereas caterpillars, mice, and fruit flies would have to be injected with the fungus. The upkeep for worms is simple, and it is possible to maintain stocks for experiments.

The Dar assay (Jain et. al., 2009; Gravato-Nobre, 2005) is one of the few assays used to survey infections in *C. elegans*. It does not require the death of worms; infection is based upon the appearance of the deformed anal region (Dar) upon ingesting *C. albicans* present on an agar plate, mixed with the standard diet of attenuated *E. coli OP50*. While this is a fairly short assay of four to six days and an indication of whether or not a worm has been infected, Dar can be subjective and difficult to identify.

In order to definitively distinguish particular immune responses or the lack thereof, the Dar assay must be followed by a more vigorous survival assay. In the survival assay, a population of worms is tracked from the start of infection until death. The current survival assay protocol requires transferring parents away from their progeny every other day – a time-intensive task. Worms must be counted every day as well. When counting, worms must be classified as alive, dead or censored if they die of unrelated causes such as dehydration.

The main objective for this project is to optimize the assay in order to not have to transfer the worms every other day and to make it more manageable. By switching to bigger plates and using sterile worms, transferring should only have to occur every couple of days. Ideally, worms should only have to be transferred every three to four days. By optimizing and validating this

assay, potential antifungal drugs can simply be added to the food spot and compared to a control plate to directly visualize their effects on the survival of *C. elegans*.

In order to streamline this process, we propose the use of the sterile mutant *fer-1* in the survival assays as it is not able to reproduce at 25^{o}C. A mutation in *fer-1* prevents the spermatids' membranous organelles from fusing with the plasma membrane and from forming short pseudopods, causing immobility (Achanzar, 1997). This stops sperm from penetrating into an egg which will prevent fertilization and the laying of eggs, the reason that the worms have to be transferred so frequently. Instead, the hermaphroditic worms lay unfertilized oocytes, which are noticeable because they are round, compared with the oval-shaped fertilized eggs (McCarter, 1999). Along with switching to the sterile mutants, the *Candida* spot was spread to increase the counter's ability to see through the dark spot. Additionally, larger 100mm plates were utilized in order to better allow for the spreading of the spot. Finally, we validated our methods using both known *Candida* mutants lacking virulence factors as well as the antifungal drug fluconazole.

Materials and Methods

Strains, Media and Growth Conditions

The mutant *C. elegans* strain *fer-1* was obtained from the Caenorhabditis Genetics Center (MN) and stocks were maintained on nematode growth medium (NGM) on *Escherichia coli* OP50. *E. coli* OP50 was cultured overnight in 5mL Luria broth at 37ºC and *Candida albicans* strains were cultured overnight in 3mL YPD at 30ºC.

Egg Preparation

After three days of growth on NGM at 20ºC, *C. elegans* were washed off with M9 buffer, transferred to a 15 mL tube and centrifuged at 2000 rpm for 2 min. The supernatant was removed by aspiration and 15 mL bleach treatment (15 mL DI water, 5 mL bleach, 2 pellets NaOH) was added. The tube was mixed gently by inverting for 3 min then centrifuged at 3000 rpm for 2 min. The supernatant was removed by aspiration, 15mL of M9 were added and the suspension was centrifuged once more at 3000 rpm for 2 min. The supernatant was again aspirated and then resuspended in M9 to obtain about 30 eggs/5 µl.

Dar Assay

Desired *Candida* strains and *E. coli* were grown overnight. 200µl of *Candida* cultures and 1.5mL of *E. coli* culture were centrifuged at full speed for 1 min in a microcentrifuge, washed twice with sterile water, and resuspended to obtain *Candida* cultures of 10 mg/mL and *E. coli* of 200 mg/mL. NGM plates were spotted with a mixture of 10µl of 50 mg/mL streptomycin, 7µl sterile water, 2.5µl *E.coli* and 0.5µl of *Candida* for each *Candida* strain in triplicate. *C. elegans* eggs were prepared and distributed on the plates. The number of worms showing the Dar phenotype as previously described (Jain, 2009) was counted and the percentage was calculated for days 3,4, and 5.

Pathogenesis Survival Assay

Desired *Candida* strains and *E. coli* were grown overnight. 200µl of *Candida* cultures and 1.5mL of *E. coli* culture were centrifuged at full speed for 1 min in a microcentrifuge, washed twice with sterile water, and resuspended to obtain *Candida* cultures of 10 mg/mL and *E. coli* of 200 mg/mL. NGM plates were spotted with a mixture of 1 µl of 50 mg/mL streptomycin, 7µl sterile water, 2.5µl *E.coli* and 0.5µl of *Candida* for each *Candida* strain in triplicate. *N2 C. elegans* eggs were prepared and distributed on 6cm NGM agar plates, and the survival of the worms was monitored by counting the live worms, dead worms, and worms on the rim of the plate each day. Worms were transferred to new plates every other day to isolate the original generation only, and overnight cultures were prepared for the next food spot on the same day as transferring. On days opposite transferring and starting overnight cultures, plates were spotted and incubated overnight at 30 ºC. This process was continued until no worms were left alive. Survival of worms was plotted using SSPS software and the p-values and chi^2 values were determined using the program to determine statistical significance between infected and uninfected *C. elegans.*

Survival Assay Optimization

Alterations to the pathogenesis survival assay were tested by using *fer-1 C. elegans* instead of the wildtype *N2* strain, spreading the food spot of *E. coli* and *C. albicans* with a large pick after overnight incubation and before transferring worms, testing on large 10cm agar plates instead of small 6cm plates, lengthening the time between transfers to three to four days, and altering the concentration of *C. albicans* in the food spot.

Survival Assay Validation

The optimized survival assay was performed with known avirulent *C. albicans* mutants, *cph1*Δ/Δ, *efg1*Δ/Δ and *cph1*Δ/Δ*efg1*Δ/Δ. The assay was then performed with wildtype SC5314

Candida in the presence of fluconazole. Using a fluconazole stock solution of 0.5 mg/mL, concentrations of 50µM, 100µM, and 150µM were created in DMSO. The fluconazole solution replaced 5µL of water within the food spot.

C. elegans RNA Extraction

100 worms were picked off of agar plates infected with *C. albicans* and put in ice cold M9 in a 1.5mL tube. Worms were washed off of two plates infected with *C. albicans* and one uninfected plate with M9 buffer. Worms washed off were spun at 3000 rpm for 5 min. and transferred to a 1.5mL tube. All tubes were spun at 13,000 rpm for 1 min. 100µl Trizol was added, mixed well, and incubated at RT for 10 min. Five freeze-thaw cycles with liquid nitrogen and a 37ºC water bath were done until no intact bodies were left. 50µl chloroform was added, tubes were mixed, and spun at 14,000 rpm for 20 min. at 4ºC. Supernatants were transferred to new tubes, 6µl glycogen was added, mixed well, and then mixed with 1 volume of isopropanol. Tubes were spun at 14,000 rpm for 20 min. at 4ºC to pellet RNA. Supernatants were removed and the pellets washed in 100µl ethanol. Tubes were spun at 7,500rpm for 5 min. at 4ºC. Ethanol was removed and pellet was left to air dry for 5 min. RNA pellet was resuspended in 10µl Nuclease-free water. RNA concentration was measured with NanoDrop2000.

RT-PCR

4µl *C. elegans* RNA was mixed with 4µl qScript cDNA SuperMix and 2µl Nuclease-free water. Tubes were incubated at 25ºC for 5 min., 42ºC for 30 min., and 85ºC for 5 min. cDNA concentrations were measured with NanoDrop2000 and 3.5µl cDNA was mixed with 2.5µl buffer, 2µl dNTPs, 0.143µl Taq polymerase and 17.86µl Nuclease-free water. cDNA was amplified by the following PCR steps: 95ºC for 2 min., 94ºC for 15 sec., 55ºC for 30 sec., 72ºC for 1 min, 30 more cycles of those four steps, 72ºC for 10 min., hold at 4ºC. Agarose (1.5%) was

added to 150mL 1x TBE running buffer and heated until the mixture dissolved. 10μL ethidium bromide was added. The gel was poured and left to dry. 1x loading dye was added to PCR samples, and 15μL of samples were loaded. The gel was run for two hours at 120V.

Results

Establishing *fer-1* as a replacement for *N2*

In order to develop a simpler, less labor-intensive survival assay, we hypothesized that infertile *C. elegans* mutants *fer-1* and *fer-15* would allow us to transfer worms less frequently. These worms are infertile at 25°C because a mutation in their spermatids prevents them from penetrating oocytes, which significantly reduces problems with eggs or larvae (Achanzar 1997).

The Dar Assay

To reinforce that *fer-1* behaves like *N2* wildtype worms in all respects except for fertilization, the wildtype and mutant strains were analyzed in terms of the Dar phenotype previously detected for *C. elegans* infected with *C. albicans* (Jain, 2009). *N2* and *fer-1* worms were used in the Dar assay. As seen in Table 1 and Figure 6, both *N2* and *fer-1* show reduced Dar when fed avirulent *C. albicans* mutants compared to the cognate wildtype. It can also be observed that *cph1*Δ/Δ has a higher percentage of Dar than either *efg1*Δ/Δ or the double mutant. The double mutant causes Dar in the lowest percentage of worms, sometimes zero percent.

Table 1: The percentage of Dar for C. elegans infected with different strains of C. albicans decreases with C. albicans of lowered virulence compared with wildtype.

The percentage of worms with Dar was calculated by counting the amount of worms with Dar and dividing that by the total live worms on the agar plate. Three Dar assays were carried out with four strains of *C. albicans* so values for the three assays were averaged and standard deviation was calculated. *Dar was not observed and counted on Day 3 for Assay 3 so two values were averaged. Panel A was done with *N2* worms ($p=1.59x10^{-6}$), Panel B was done with *fer-1*($p=1.46x10^{-6}$).

A

Day	*caf2-1*	*cph1*Δ/Δ	*efg1*Δ/Δ	*cph1* Δ/Δ*efg1*Δ/Δ
3*	62% +/- 9	35% +/- 19	9% +/- 1	1% +/- 1
4	72% +/- 15	35% +/- 15	10% +/- 0	3% +/- 3
5	84% +/- 14	29% +/- 6	20% +/- 5	4% +/- 2

B

Day	*caf 2-1*	*cph1*Δ/Δ	*efg1*Δ/Δ	*cph1*Δ/Δ*efg1*Δ/Δ
3	46 +/- 4	49 +/ 12	8 +/- 3	0 +/- 0
4	90 +/- 9	89 +/ 21	22 +/ 2	7 +/- 3
5	100 +/- 0	88 +/ 14	25 +/- 6	11 +/- 4

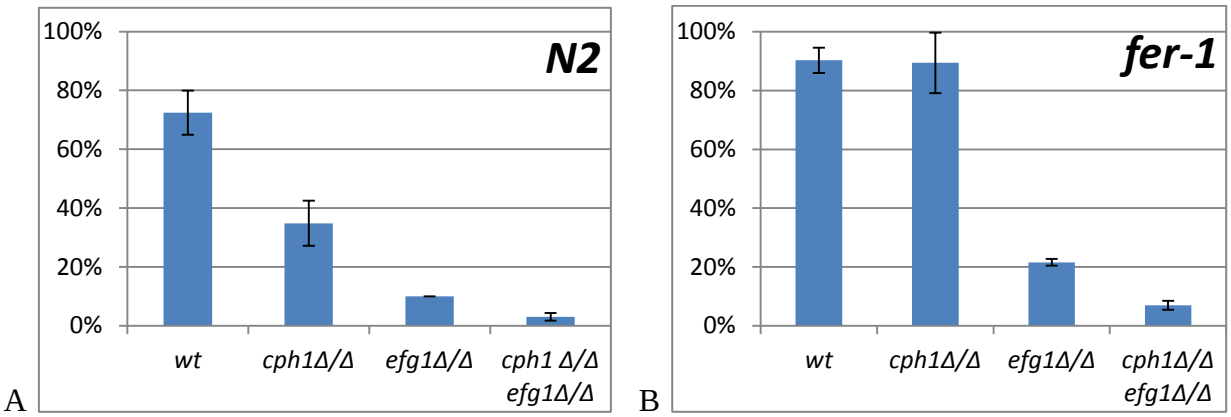

Figure 6: Mutant C. albicans elicit less Dar in C. elegans than wildtype.
Average percentages of the three Dar assays for Day 4 were graphed with the previously identified avirulent strains of *C. albicans*. Panel A Dar assay was performed with *N2* wildtype worms, Panel B Dar assay with *fer-1*.

Optimizing the Survival Assay

To measure the lifespan of worms exposed to *C. albicans*, the infected population, 60 *N2* worms, must be separated from their progeny daily. This process must be continued every day until the worms succumb, and is lengthy and time-consuming. In addition to using *fer-1*, other alterations to streamline the assay were attempted and instituted if useful. The optimization protocol's possible stepwise experimental solutions are presented in Figure 7. Using infertile mutants and the optimization steps allowed for the transfer of worms every three to four days.

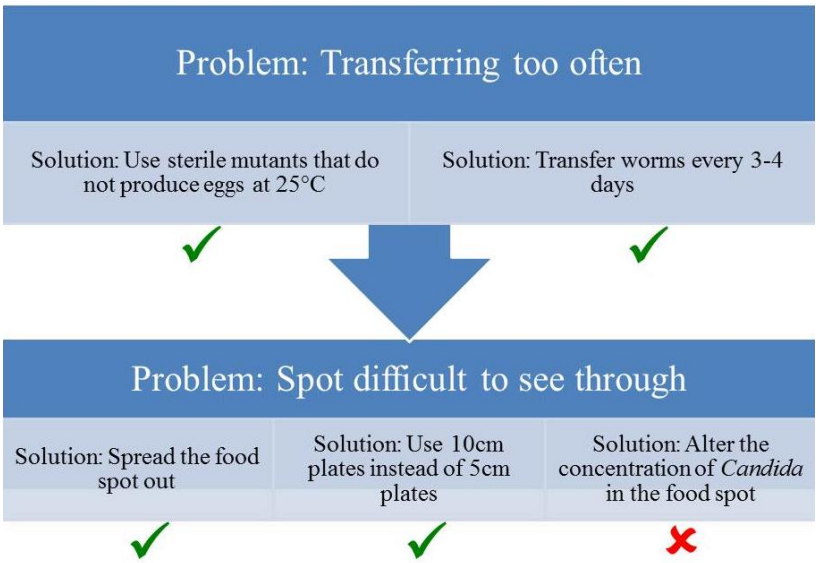

Figure 7: Survival Assay Optimization Process

The survival of the two mutant strains, both *fer-1* and *fer-15*, parallel that of *N2* wildtype worms when infected with *C. albicans* – worms fed *C. albicans* die more quickly as shown in Figure 8 ($p = 0.000$) (Pukkila-Worley, 2011). The mutant strains behave comparably to wildtype except that their lifespan is slightly shorter under optimal conditions, feeding on *E. coli* attenuated with streptomycin. In optimizing the new survival assay, *fer-1* was the more reliable strain – the *fer-15* was leaky as a result of any slight derivation from the restrictive temperature. For example, room temperature when transferring worms would allow them to lay eggs which interfered with the survival assay. The life span analysis of *fer-15* was similar to that of *fer-1* (data not shown, $chi^2=72$, $p=0.000$).

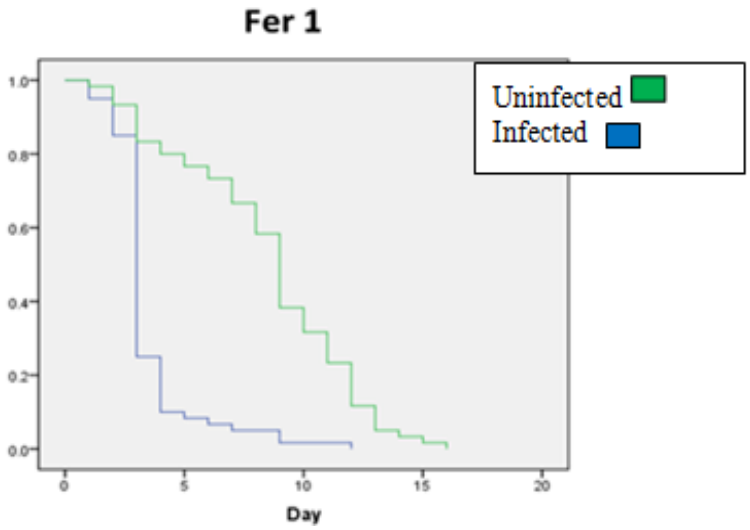

Figure 8: *Fer-1 C. elegans* mutants survive longer when they are not fed *C. albicans*.
The day of the survival assay is indicated on the x-axis and the percentage of C. elegans alive on that day is indicated on the y-axis. Green lines indicate worms fed only dead E. coli ("uninfected") and blue lines represents "infected" worms fed live C. albicans in addition to dead E. coli. For all assays, n=60 ($chi^2 = 51.313$, $p = 0.000$).

In order to determine if *fer-1* reacted similarly to *N2* in response to known avirulent mutants, validating the altered survival assay, two mutant survival assays were performed (Figure 9). In this case, the double mutant *cph1*Δ/Δ*efg1*Δ/Δ was avirulent (Figure 9A). *Efg1*Δ/Δ showed reduced virulence compared to wildtype. Interestingly, *cph1*Δ/Δ showed very similar results to the wildtype strain. These data are similar to those of *N2*, indicating that the sterile

mutants are parallel to *N2* and are an appropriate substitute. Furthermore, the degree of pathogenesis between the mutants in the two strains of *C. elegans* match pathogenicity analyses comparing wildtype *C. albicans* to potentially avirulent mutants in the mouse model (Lo, 1997).

The second mutant validation survival assay (Figure 9B) revealed no noticeable difference between the wildtype *SN250* strain and the mutants *zcf15*Δ/Δ and *orf19.1219*Δ/Δ. The products of these two genes have previously been established as components of the *C. albicans* pathogenesis pathways using the Dar assay as a measure of pathogenicity (Pastor, 2010).

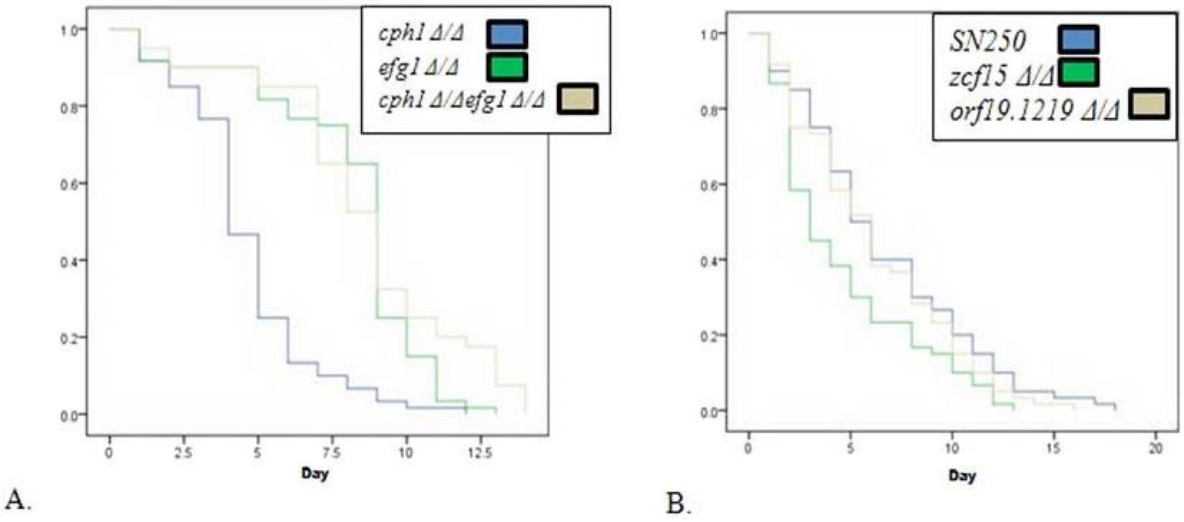

Figure 9: Validation of mutant survival assay with known C. albicans mutants with reduced virulence. The day of the assay is indicated on the x-axis and the percentage of C. elegans alive on that day is indicated on the y-axis. The lines represent the percentage of *C. elegans* alive on that day is indicated on the y-axis. A. The blue line represents the percentage living on agar plates with *C. albicans cph1Δ/Δ*, the green line represents plates with *efg1Δ/Δ* and the tan line represents plates with *cph1Δ/Δ efg1Δ/Δ* (chi^2 = 63.151, p = 0.000). B. The blue line represents plates with wildtype *C. albicans* strain *SN250*, the green line represents plates with *zcf15Δ/Δ*, and the tan line represents plates with *orf19.12.19Δ/Δ* (chi^2 =10.759, p=0.005).

As a result of growing the *C. albicans* for three or four days at a time, the food spot overgrew and was difficult to see through. This made getting an accurate count of worms difficult. In order to increase visibility through the spot, three different techniques were used, as detailed in the assay optimization flowchart, Figure 7. First, the food spot was spread over a wider area on the plate with a blunt wire pick before transferring worms onto the plate. Another

technique employed was the use of 10 cm plates instead of 5 cm plates. These two techniques were tested both individually and together by spotting plates, placing them in the 30°C incubator overnight, spreading the appropriate plates the next day, and then putting the plates in the 25°C incubator for four days – mimicking the time between transfers. After four days, the plates were removed from the incubator and observed. The spread 10 cm plate had the most transparent spot. Figure 10 shows both the original and implemented techniques after four days at 25°C both as a plate on the bench and under the microscope, zoomed in on the spot. *C. elegans* worms in the spot are marked by arrows. Because of the increased transparency of the spot, spreading and 10 cm plates were used in all future assays.

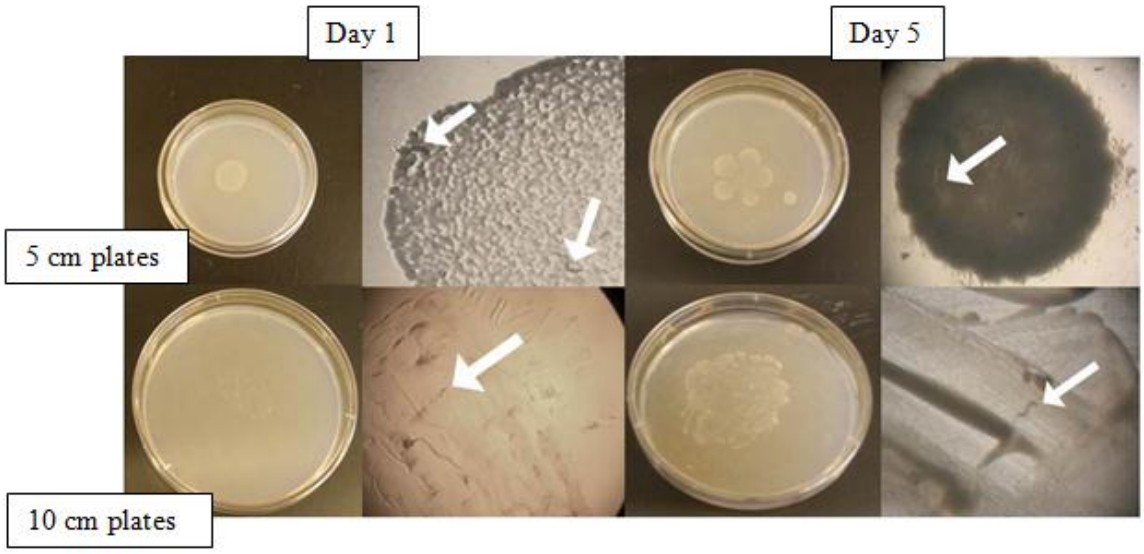

Figure 10: Opacity of the C. albicans food spot
From left to right, day 1 of the plate, day 1 under a dissecting microscope at 5X, day 5 of the plate, day 5 under a dissecting microscope at 5X. The top row is the 5 cm plate with an un-spread spot. The bottom row is a 10 cm plate, with the food spot spread out. White arrows indicate *C. elegans*.

In order to increase visibility of the worms further, decreased concentrations of *C. albicans* were used in the food spot. The three concentrations used were 10 mg/mL (original), 5 mg/mL and, 2.5 mg/mL. As can be seen in Figure 11, there was no significant difference in the life span of the worms on the different concentrations. From observation, the spot was no more

opaque in the 2.5 mg/mL plates as the 10 mg/mL plates, as depicted in Figure 10. Worms in the *Candida* spot are clearly visible, and the spot itself is transparent, whether viewed with the naked eye or under a microscope. Thus, a different concentration of *C. albicans* was not implemented in the new survival assay optimized for the infertile *C. elegans* mutants.

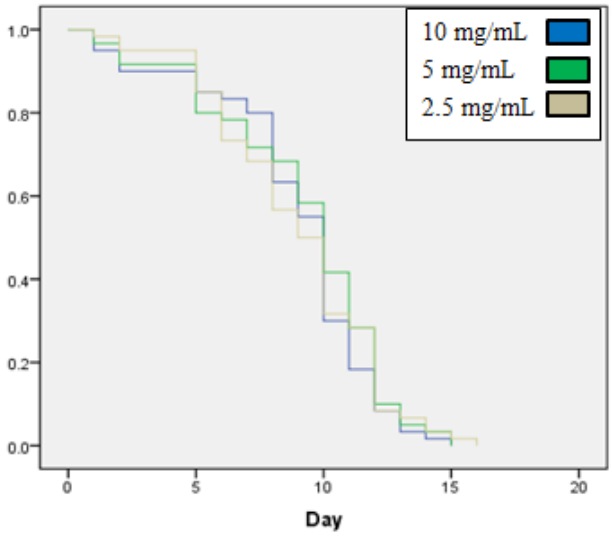

Figure 11: Survival Assay to Optimize of *C. albicans* concentration reveals no significant difference in infection with *C. albicans* at 10, 5 and 2.5mg/mL

The day of the assay is indicated on the x-axis and the percentage of *C. elegans* alive on that day is indicated on the y-axis. The blue line represents the percentage living on agar plates with 0.5µl of 10 mg/mL *C. albicans*, the green line represents plates with 0.5µl of 5 mg/mL and the tan line represents plates with 0.5µl of 2.5 mg/mL ($Chi^2 = 0.579$, $p = 0.749$).

Fer-1's response to fluconazole

To validate that *fer-1* worms respond to antifungal drugs in the survival assay like wildtype *N2* worms, titrations of fluconazole were incorporated into the food spot. Worms given 150 µg/mL, 100 µg/mL, and 50 µg/mL of fluconazole when infected with *C. albicans* were compared with worms not given any drug, depicted in Figure 12. Overall, the worms lived longest on the 50 µg/mL fluconazole, with the exception of a couple of worms at the end of the survival. Not only did worms live slightly longer in the presence of fluconazole, but the *Candida* was visibly less pathogenic. It became lighter in color, clumpier, and did not form a uniform lawn on the agar.

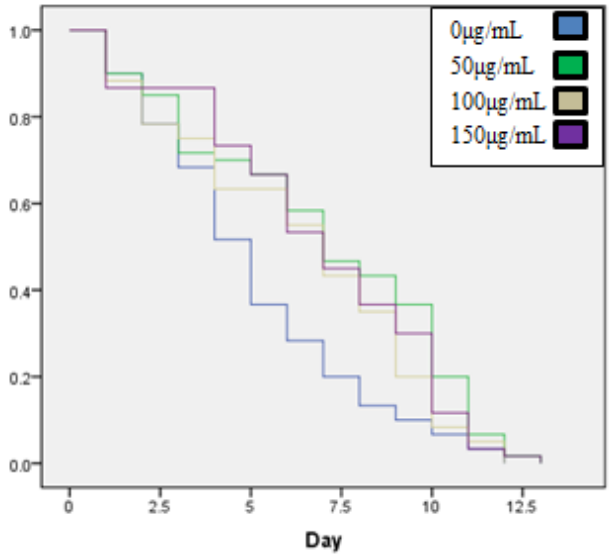

Figure 12: Survival assay for the titration of fluconazole as an antifungal drug

The day of the assay is indicated on the x-axis and the percentage of *C. elegans* alive on that day is indicated on the y-axis. The blue line represents the percentage of worms living on control plates with 0 µg/mL fluconazole, the green line represents plates with 50 µg/mL fluconazole, the yellow line represents plates with 100 µg/mL fluconazole, and the purple line represents plates with 150 µg/mL (Chi^2 = 12.784, p = 0.005)

Peptides of the *C. elegans* innate immune system

In order to further validate that the survival assay is measuring the pathogenesis of the fungus and not other factors such as contamination or dehydration, semi-quantitative RT-PCR was performed on both *C. elegans* infected with *Candida* and uninfected, living only on dead *E. coli.* The mRNA transcripts amplified, the genes encoding NLP29, NLP31, ABF2, IRG1 and HSP16.41, were chosen because they have been found to be upregulated during *C. elegans* infection. NLP29 and NLP31 are antimicrobial peptides (AMPs) known to be a part of the *C. elegans* immune response as a part of the MAP kinase pathway, and can be turned on by *D. coniospora* infection (Pujol, 2008). ABF2 is an AMP upregulated in the response against many pathogens, but especially upon fungal infection from *C. krusei*, *S. cerevisiae*, *T. delbrueckii* and *D. hansenii*, among others (Kato, 2002). IRG-1 is a host "infection response gene" also a part of the MAP kinase pathway and known to be induced by *P. aeruginosa* infection (Estes, 2010) and

HSP16.41 is a heat shock protein implicated in the innate immune response against *Salmonella enterica* (Tenor, 2008).

As seen in Figure 13, the samples from infected worms had significantly darker bands from AMP transcripts upon electrophoresis compared with uninfected worms. The actin control was transcribed at the same level by the two groups of worms (ACT1) but the AMP transcripts for NLP29, NLP31, and ABF2 seemed to be upregulated in infected worms. There is only a very faint band in the infected lane of HSP16.41, and IRG1 did not appear to be upregulated upon infection (data not shown). These data suggest that these two proteins may not be a significant part of the innate immune system pathways, but that NLP29, NLP31 and ABF2 are turned on in these pathways.

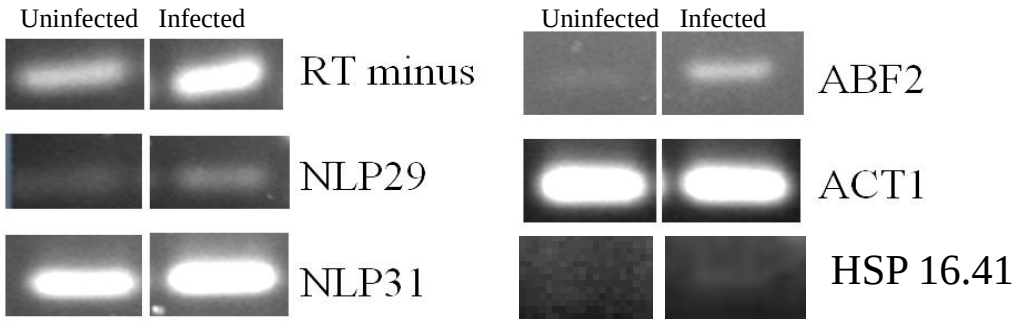

Figure 13: RT-PCR of *C. elegans* infected with *C. albicans* versus uninfected shows higher levels of antimicrobial peptide RNA upon infection

Gel electrophoresis of cDNAs after reverse transcription from RNAs extracted from uninfected and infected worms. RT minus is Actin RNA before reverse transcription. NLP29, NLP31 and ABF2 are antimicrobial peptides and HSP16.41 is a Heat Shock Protein. ACT1 is the Actin gene 1 transcribed from cDNA.

Discussion

We have demonstrated that the use of sterile *C. elegans* is a viable alternative to wildtype for analyzing the lifespan of worms infected with *C. albicans*. *Fer-1* mutants were used because they do not produce viable eggs. This means that with the use of sterile worms, transferring the original infected worms away from their progeny, which is necessary to measure the life-span, was not necessary. The modified protocol requires transfer of worms to agar plates when the *C. albicans* lawn has grown too thick to see the worms to count which are alive and dead, approximately every four days. In order to maximize the amount of time between transfers, large 10cm agar plates were used which allowed the food spot out to be as spread out as possible.

Our studies show that *fer-1* is a suitable substitute for wildtype *N2* worms on several accounts. First, the sterile worms have a comparable lifespan to wildtype, for both worms infected with *C. albicans* in addition to uninfected control worms. Secondly, when infected with previously identified avirulent mutants such as *cph1*Δ/Δ*efg1*Δ/Δ, *cph1*Δ/Δ and *efg1*Δ/Δ, *fer-1* Dar patterns matched those of *N2*. These data correctly correlate with the mutants missing virulence factors implicated in the formation of hyphae. In the survival assay with the same three mutants, the worms on *cph1*Δ/Δ*efg1*Δ/Δ lived the longest and their lifespan matched that of uninfected worms. The worms on *efg1*Δ/Δ were similar to that of uninfected worms, but the lifespan of these worms was slightly shorter. In the survival curve using *cph1*Δ/Δ, worms died faster than the other two mutant strains and appeared sicker. They moved slowly if at all, and had distended abdomens. This further validated the assay because the *fer-1* survival with these mutants matches similar studies in mice (Lo, 1997). Thus, new avirulent mutants can be discovered using this pathogenesis survival assay in a mutant screen, in order to give rise to genes that are a part of *Candida* pathogenesis pathways. The mutant screen would be easier and

less labor-intensive than a screen using wildtype *C. elegans* or a mammal such as mice as a result of the optimized survival assay. The *C. elegans* only live for about two to three weeks, while mice live much longer, which will allow for more screens to take place in a given amount of time, with a given amount of technicians, time and money.

While a lifespan of two to three weeks is simpler and more efficient than a longer lifespan, evaluating the pathogenicity of *C. albicans* in just one week is less reliable. The Dar assay, observing enlarged anal regions in worms infected with *C. albicans*, is five days long, and does not give a large scope of the effects of the infection like a survival assay does. As seen in all of the survival curves in this study, there is a large drop-off of live worms between day five and ten, marking a switch in the *Candida* infection thriving and the worms' immune systems succumbing to the disease. The shorter Dar assay may not take this event into account. Furthermore, it is quite unambiguous and objective to determine if a worm is alive or dead, but the Dar phenotype is much more subjective to observe. Sometimes the Dar phenotype can be difficult to see, especially for worms within the *Candida* lawn, and there are often worms that are "borderline," and their anal region could equally be deformed or normal. Therefore, our data and observations propose this survival assay a much more reliable measure of *C. albicans* pathogenesis.

It was found that leaving sterile worms out of the 25 ºC incubator for more than a few minutes at a time exacerbated the issue of the leaky *fer-1* and *fer-15* mutations. It is very important to know what the precise room temperature of a lab is, rather than generalizing what the room temperature is approximately supposed to be. One problem with *fer-1* and *fer-15* is that the sterility mutation was leaky. Slight changes in temperature, even the short time to transfer, caused *fer-15* to lay eggs. While there were not many larvae, it caused enough of a problem to

discontinue its use in further studies. *Fer-1* was leaky from the time the eggs were plated, in order to begin a survival assay, until the first transfer. After the first transfer, there were no more eggs being laid. During the time before the first transfer, any larvae found were killed, allowing the first generation to still be distinguished from newer ones.

The concentrations of antifungal drug used in the fluconazole survival assay ranged from a control plate (0uM) to 150uM in 50uM increments. The 50uM concentration of fluconazole was the most effective for maintaining the health of the worms. A contributing factor may have been that there was some contamination on the 100 and 150uM plates. Once a transfer occurred, the contamination disappeared.

Even though there were higher concentrations used than 50uM, they appeared to not be as effective in increasing the survival of the worms. It is probable that the death rate of the worms on food spots containing 100 and 150uM fluconazole was influenced by the harmful effects of fluconazole on the host. Fluconazole attacks ergosterol, which is very similar to cholesterol – contained in animal cell membranes. These concentrations of fluconazole may have simply been too high for the 1mm nematodes. In order to account for this in future studies, a survival assay giving worms only the drug should be completed to determine the drug's natural effects on the worms' lifespan. In order to use this assay for other potential drugs, similar assays must be completed. First, a survival must be carried out with only the possible antifungal drug to establish if it negatively affects worms, and a second with *C. albicans* and the possible antifungal drug to determine its ability to either halt or prevent infections in *C. elegans*. Another useful step would be to apply the optimum concentration of drug before infecting the worms, at the same time as infecting the worms (the option used in this study) and after infecting the worms, and evaluate various survival rates.

One of the results seen in the survival assay with fluconazole was that the *C. albicans* did not die, but became a significantly different consistency – clumpier and darker. Fluconazole is fungistatic which means that it will not kill the fungus; it inhibits its growth and replication. The change in phenotype was thought to be due to the inhibited growth but not death of the *C. albicans*. There are fungicidal – fungus killing – antifungal medications, but these tend to be more toxic to the host, such as Amphotericin B. One problem with fungistatic medications is that the small amount of fungus left over can proliferate and potentially become resistant to the antifungal drug or compound. This has been a serious problem with antifungal drugs, because more and more strains of *C. albicans* and other fungi are becoming resistant to fungistatic antifungal medications.

Semi-quantitative RT-PCR was performed on infected and uninfected *C. elegans* in order to verify that an antifungal immune response was taking place upon infection with *C. albicans*. As depicted by the RT minus bands, there was some genomic DNA contamination in the RNA extraction. Because some DNA was isolated as well, the levels of the genes amplified by PCR are not as high as the bands are bright – in other words, the brightness of the "RNA" before reverse transcription bands must be subtracted from the RT-PCR bands. This makes for a less drastic difference in viewing the upregulation of transcripts of antimicrobial peptides during fungal infection. However, the cDNAs of the uninfected and infected Actin-1 gene are identical, indicating that in an unrelated gene, the amount of mRNA expressed was the same for both uninfected and infected worms, and giving validity to the experiment.

There were three AMP transcripts that seemed to be upregulated upon infection with *C. albicans*: NLP-29, NLP-31, and ABF-2. This data indicates that these peptides may have a distinct role in the innate immune system against fungal infection within *C. elegans*, elucidating

a better understanding of those particular molecular pathways. There was no IRG-1, a host response gene (data not shown), and very little HSP16.41 mRNA present in order to make cDNA during RT-PCR. This may indicate that not only are these proteins not upregulated during infection, but are also not produced at this particular time in the life cycle of the nematodes (RNA isolated at 72 hours). This procedure has been done with wildtype *N2* worms and no conclusive upregulation of AMP transcripts was detected (Luca Issi, personal communication). Further experiments must be done to test both *N2* worms and *fer-1* mutants at earlier time points than at day 3, or 72 hours post-infection, to definitively determine if and when these AMP transcripts are produced. Regardless, these findings are important because the immune system of *C. elegans* is very similar to the innate immune system of humans, with comparable if not homologous pathways. Therefore, the more information gained about the *C. elegans* immune system, the more knowledge gained about the innate immune system in humans.

Appendix

In this appendix are all of the raw data worksheets used to mark live, dead or "edge" worms throughout survival experiments. Worms that are marked by "edge" died for reasons other than *Candida* infection, such as dehydration on the edge of the agar plate or digging into the agar and not resurfacing. The worksheets are in this order: *fer-1* and *fer-15* trials to establish lifespan when infected and uninfected, validation with known avirulent mutants *cph1*Δ/Δ, *efg1*Δ/Δ and *cph1*Δ/Δ*efg1*Δ/Δ, validation with potential avirulent mutants *zcf15*Δ/Δ and *orf19.1219*Δ/Δ compared with wildtype *SN250*, the *Candida* concentration titration, and the fluconazole concentration titration.

UNINFECTED		**Strain: Fer1**	**Plate A**			**Plate B**		
	Date	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3		start cultures (E.coli)						
Day -2		egg prep + spotting, start cultures						
Day -1		spotting						
Day 0	10/31/2011	transfer same # (20) of worms,	25	0	0	26	0	0
Day 1	11/1/2011	count (edge v. dead)	13	7	5	19	4	3
Day 2	11/2/2011	count (edge v. dead), start cultures	13	0	0	19	0	0
Day 3	11/3/2011	count (edge v. dead), spot	12	0	1	19	0	0
Day 4	11/4/2011	transfer (edge v. dead*)	12	0	0	19	0	0
Day 5	11/5/2011	count (edge v. dead)	11	1	0	17	1	1
Day 6	11/6/2011	count (edge v. dead) start cultures	10	0	1	17	0	0
Day 7	11/7/2011	count (edge v. dead), spot	10	0	0	17	0	0
Day 8	11/8/2011	transfer (edge v. dead)	6	1	3	6	0	11
Day 9	11/9/2011	count (edge v. dead), start cultures	5	0	1	3	0	3
Day 10	11/10/2011	count (edge v. dead), spot	0	0	5	0	0	3

INFECTED		**Strain: Fer1**	**Plate A**			**Plate B**			**Plate C**		
	Date	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	10/31/2011	transfer same # (20) of worms,	15	0	0	23	0	0	20	0	0
Day 1	11/1/2011	count (edge v. dead)	9	3	3	16	2	5	12	0	8
Day 2	11/2/2011	count (edge v. dead), start cultures	9	0	0	15	0	1	9	1	2
Day 3	11/3/2011	count (edge v. dead), spot	5	1	3	11	1	3	6	0	3
Day 4	11/4/2011	transfer (edge v. dead*)	4	0	1	8	3	0	3	1	2
Day 5	11/5/2011	count (edge v. dead)	3	0	1	3	2	3	3	0	0
Day 6	11/6/2011	count (edge v. dead) start cultures	2	0	1	3	0	0	2	0	1
Day 7	11/7/2011	count (edge v. dead), spot	2	0	0	2	0	1	2	0	0
Day 8	11/8/2011	transfer (edge v. dead)	1	0	1	1	0	1	2	0	0
Day 9	11/9/2011	count (edge v. dead), start cultures	1	0	0	1	0	0	1	0	1
Day 10	11/10/2011	count (edge v. dead), spot	1	0	0	1	0	0	1	0	0
Day 11	11/11/2011	transfer (edge v. dead)	0	0	1	0	0	1	0	0	1

UNINFECTED		Strain: Fer-1	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	12/1/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	12/2/2011	count (edge v. dead)	19	0	1	20	0	0	20	0	0
Day 2	12/3/2011	count (edge v. dead), start cultures	18	1	0	19	0	1	19	0	1
Day 3	12/4/2011	count (edge v. dead), spot	18	0	0	14	5	0	18	1	0
Day 4	12/5/2011	transfer (edge v. dead*)	16	1	1	14	0	0	18	0	0
Day 5	12/6/2011	count (edge v. dead)	14	0	2	14	0	0	18	0	0
Day 6	12/7/2011	count (edge v. dead) start cultures	14	0	0	12	2	0	18	0	0
Day 7	12/8/2011	count (edge v. dead), spot	13	0	1	10	1	1	17	0	1
Day 8	12/9/2011	transfer (edge v. dead)	13	0	0	9	0	1	13	1	3
Day 9	12/10/2011	count (edge v. dead), start cultures	8	0	5	4	0	5	11	0	2
Day 10	12/11/2011	count (edge v. dead), spot	5	2	1	3	0	1	11	0	0
Day 11	12/12/2011	transfer (edge v. dead)	3	0	2	1	0	2	10	0	1
Day 12	12/13/2011	count (edge v. dead), start cultures	3	0	0	1	0	0	3	0	7
Day 13	12/14/2011	count (edge v. dead), spot	2	0	1	0	0	1	1	0	2
Day 14	12/15/2011	transfer (edge v. dead)	1	0	1	0	0	0	1	0	0
Day 15	12/16/2011	count (edge v. dead)	1	0	0	0	0	0	0	0	1
Day 16	12/17/2011	count (edge v. dead) start cultures	0	0	1	0	0	0	0	0	0

INFECTED		Strain: Fer-1	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	12/1/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	12/2/2011	count (edge v. dead)	19	0	1	19	0	1	19	0	1
Day 2	12/3/2011	count (edge v. dead), start cultures	19	0	0	16	0	3	16	0	3
Day 3	12/4/2011	count (edge v. dead), spot	7	0	12	7	0	9	1	0	15
Day 4	12/5/2011	transfer (edge v. dead*)	3	0	4	3	0	4	0	0	1
Day 5	12/6/2011	count (edge v. dead)	3	0	0	2	0	1	0	0	0
Day 6	12/7/2011	count (edge v. dead) start cultures	3	0	0	1	0	1	0	0	0
Day 7	12/8/2011	count (edge v. dead), spot	2	0	1	1	0	0	0	0	0
Day 8	12/9/2011	transfer (edge v. dead)	2	0	0	1	0	0	0	0	0
Day 9	12/10/2011	count (edge v. dead), start cultures	1	0	1	0	0	1	0	0	0
Day 10	12/11/2011	count (edge v. dead), spot	1	0	0	0	0	0	0	0	0
Day 11	12/12/2011	transfer (edge v. dead)	1	0	0	0	0	0	0	0	0
Day 12	12/13/2011	count (edge v. dead), start cultures	0	0	1	0	0	0	0	0	0

UNINFECTED		Strain: Fer-15 on E.coli	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -1	9/29/2011	start cultures									
Day 0	9/30/2011	egg prep + spotting									
Day 1	10/1/2011	start cultures									
Day 1.5	10/2/2011	spotting and counting									
Day 2	10/3/2011	transfer same # of worms, start culture	30	0	0	30	0	0	30	0	0
Day 3	10/4/2011	spotting, count (edge v. dead)	29	1	0	30	0	0	28	0	2
Day 4	10/5/2011	transfer, (edge v. dead*), start culture	28	1	1	29	1	0	24	2	2
Day 5	10/6/2011	spotting, count (edge v. dead)	25	1	2	27	1	1	24	0	0
Day 6	10/7/2011	transfer, (edge v. dead*), start culture	25	1	2	19	1	7	23	0	1
Day 7	10/8/2011	spotting, count (edge v. dead)	24	0	1	19	0	0	24	0	1
Day 8	10/9/2011	transfer, (edge v. dead*), start culture	24	0	0	19	0	0	21	0	3
Day 9	10/10/2011	spotting, count (edge v. dead)	24	0	0	18	0	1	21	0	0

INFECTED		Strain: Fer-15 on E. coli and Candida	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -1	9/29/2011	start cultures									
Day 0	9/30/2011	egg prep + spotting									
Day 1	10/1/2011	start cultures									
Day 1.5	10/2/2011	spotting and counting	12	1	0	11	2	1	31	1	0
Day 2	10/3/2011	transfer same # of worms, start culture	9	0	3	11	2	0	18	5	8
Day 3	10/4/2011	spotting, count (edge v. dead)	7	0	2	4	0	7	8	2	8
Day 4	10/5/2011	transfer, (edge v. dead*), start culture	2	2	3	2	0	2	7	0	1
Day 5	10/6/2011	spotting, count (edge v. dead)	1	0	1	2	0	0	4	2	0
Day 6	10/7/2011	transfer, (edge v. dead*), start culture	0	0	1	2	0	0	4	0	0
Day 7	10/8/2011	spotting, count (edge v. dead)	0	0	0	2	0	0	4	0	0
Day 8	10/9/2011	transfer, (edge v. dead*), start culture	0	0	0	2	0	0	1	0	3
Day 9	10/10/2011	spotting, count (edge v. dead)	0	0	0	1	0	0	0	0	1
Day 10	10/11/2011	transfer, (edge v. dead*), start culture	0	0	0				0	0	0

UNINFECTED		Strain: Fer 15	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	11/4/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	11/5/2011	count (edge v. dead)	20	0	0	20	0	0	6	6	0
Day 2	11/6/2011	count (edge v. dead), start cultures	20	0	0	19	0	1	5	1	0
Day 3	11/7/2011	count (edge v. dead), spot	20	0	0	19	0	0	3	6	0
Day 4	11/8/2011	transfer (edge v. dead*)	20	0	0	17	2	0	5	1	0
Day 5	11/9/2011	count (edge v. dead)	19	1	0	17	0	0	4	0	1
Day 6	11/10/2011	count (edge v. dead) start cultures	13	1	5	14	0	3	4	0	0
Day 7	11/11/2011	count (edge v. dead), spot	10	0	3	11	0	3	3	0	1
Day 8	11/12/2011	transfer (edge v. dead)	6	1	4	7	1	3	3	0	0
Day 9	11/13/2011	count (edge v. dead), start cultures	4	0	2	4	0	3	2	0	1
Day 10	11/14/2011	count (edge v. dead), spot	3	0	1	1	0	3	1	0	1
Day 11	11/15/2011	transfer (edge v. dead)	3	0	0	0	0	1	0	0	1
Day 12	11/16/2011	count (edge v. dead), start cultures	1	0	2	0	0	0	0	0	0
Day 13	11/17/2011	count (edge v. dead), spot	0	0	1	0	0	0	0	0	0

INFECTED		**Strain: Fer 15**	**Plate A**			**Plate B**			**Plate C**		
	Date	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	11/4/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	11/5/2011	count (edge v. dead)	20	0	0	14	0	1	18	2	0
Day 2	11/6/2011	count (edge v. dead), start cultures	20	0	0	14	0	0	16	4	0
Day 3	11/7/2011	count (edge v. dead), spot	20	0	0	12	2	0	20	0	0
Day 4	11/8/2011	transfer (edge v. dead*)	20	0	0	12	0	0	13	4	3
Day 5	11/9/2011	count (edge v. dead)	18	0	2	11	1	0	11	0	2
Day 6	11/10/2011	count (edge v. dead) start cultures	12	1	5	9	1	1	10	0	1
Day 7	11/11/2011	count (edge v. dead), spot	11	0	1	9	0	1	10	0	0
Day 8	11/12/2011	transfer (edge v. dead)	5	0	6	6	0	3	6	1	3
Day 9	11/13/2011	count (edge v. dead), start cultures	3	0	2	3	0	3	2	2	2
Day 10	11/14/2011	count (edge v. dead), spot	3	0	0	3	0	0	1	0	1
Day 11	11/15/2011	transfer (edge v. dead)	1	0	2	2	0	1	1	0	0
Day 12	11/16/2011	count (edge v. dead), start cultures	0	0	1	1	0	1	0	0	1
Day 13	11/17/2011	count (edge v. dead), spot	0	0	0	0	0	1	0	0	0

		Strain: fer-1 on cph	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	2/7/2011	start cultures (E.coli)									
Day -2	2/8/2011	egg prep + spotting, start cultures									
Day -1	2/9/2011	spotting									
Day 0	2/10/2011	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	2/11/2011	spotting, count (edge v. dead)	17	3	0	20	0	0	18	2	0
Day 2	2/12/2011	transfer, (edge v. dead*), start culture	15	1	1	19	0	1	17	1	0
Day 3	2/13/2011	spotting, count (edge v. dead)	13	0	2	17	0	2	16	0	1
Day 4	2/14/2011	transfer, (edge v. dead*), start culture	13	0	0	9	2	6	6	2	8
Day 5	2/15/2011	spotting, count (edge v. dead)	4	2	7	6	0	3	5	0	1
Day 6	2/16/2011	transfer, (edge v. dead*), start culture	2	0	2	2	0	4	4	0	1
Day 7	2/17/2011	spotting, count (edge v. dead)	1	1	0	1	0	1	4	0	0
Day 8	2/18/2011	transfer, (edge v. dead*), start culture	0	0	1	1	0	0	3	0	1
Day 9	2/19/2011	spotting, count (edge v. dead)				0	0	1	2	0	1
Day 10	2/20/2011	transfer, (edge v. dead*), start culture							1	0	1
Day 11	2/21/2011	spotting, count (edge v. dead)							1	0	0
Day 12	2/22/2011	transfer, (edge v. dead*), start culture							0	0	1

		Strain: fer-1 on efg	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	2/7/2011	start cultures (E.coli)									
Day -2	2/8/2011	egg prep + spotting, start cultures									
Day -1	2/9/2011	spotting									
Day 0	2/10/2011	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	2/11/2011	spotting, count (edge v. dead)	18	0	2	17	3	0	20	0	0
Day 2	2/12/2011	transfer, (edge v. dead*), start culture	17	0	1	17	0	0	20	0	0
Day 3	2/13/2011	spotting, count (edge v. dead)	17	0	0	17	0	0	20	0	0
Day 4	2/14/2011	transfer, (edge v. dead*), start culture	17	0	0	17	0	0	20	0	0
Day 5	2/15/2011	spotting, count (edge v. dead)	15	2	0	16	1	0	18	1	1
Day 6	2/16/2011	transfer, (edge v. dead*), start culture	14	1	0	14	2	0	18	0	0
Day 7	2/17/2011	spotting, count (edge v. dead)	14	0	0	13	1	0	18	0	0
Day 8	2/18/2011	transfer, (edge v. dead*), start culture	13	1	0	13	0	0	13	0	5
Day 9	2/19/2011	spotting, count (edge v. dead)	4	0	9	6	0	7	5	0	8
Day 10	2/20/2011	transfer, (edge v. dead*), start culture	4	0	0	3	0	3	2	0	3
Day 11	2/21/2011	spotting, count (edge v. dead)	1	0	3	0	0	3	1	0	1
Day 12	2/22/2011	transfer, (edge v. dead*), start culture	1	0	0				0	0	1
Day 13	2/23/2011	spotting, count (edge v. dead)	0	0	1						

		Strain: fer-1 on cph/efg double	Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	2/7/2011	start cultures (E.coli)						
Day -2	2/8/2011	egg prep + spotting, start cultures						
Day -1	2/9/2011	spotting						
Day 0	2/10/2011	transfer same # of worms, start culture	20	0	0	20	0	0
Day 1	2/11/2011	spotting, count (edge v. dead)	20	0	0	18	0	2
Day 2	2/12/2011	transfer, (edge v. dead*), start culture	18	0	2	18	0	0
Day 3	2/13/2011	spotting, count (edge v. dead)	18	0	0	18	0	0
Day 4	2/14/2011	transfer, (edge v. dead*), start culture	18	0	0	18	0	0
Day 5	2/15/2011	spotting, count (edge v. dead)	17	1	0	17	0	1
Day 6	2/16/2011	transfer, (edge v. dead*), start culture	17	0	0	17	0	0
Day 7	2/17/2011	spotting, count (edge v. dead)	14	3	0	12	4	1
Day 8	2/18/2011	transfer, (edge v. dead*), start culture	12	0	2	9	0	3
Day 9	2/19/2011	spotting, count (edge v. dead)	7	0	5	6	1	2
Day 10	2/20/2011	transfer, (edge v. dead*), start culture	5	0	2	5	0	1
Day 11	2/21/2011	spotting, count (edge v. dead)	3	0	2	5	0	0
Day 12	2/22/2011	transfer, (edge v. dead*), start culture	3	0	0	4	0	1
Day 13	2/23/2011	spotting, count (edge v. dead)	1	0	2	2	1	1
Day 14	2/24/2011	transfer, (edge v. dead*), start culture	0	0	1	0	0	2

		Strain: fer-1 on WT SN250	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3	1/13/2011	start cultures (E.coli)									
Day -2	1/14/2011	egg prep + spotting, start cultures									
Day -1	1/15/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	16	0	4	19	0	1	19	1	0
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	15	0	1	18	0	1	18	0	1
Day 3	1/20/2011	spotting, count (edge v. dead)	10	0	5	17	0	1	18	0	0
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	7	1	2	15	0	2	16	2	0
Day 5	1/22/2011	spotting, count (edge v. dead)	6	0	1	14	0	1	10	0	6
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	6	0	0	9	0	5	9	0	1
Day 7	1/24/2011	spotting, count (edge v. dead)	6	0	0	9	0	0	9	0	0
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	3	3	0	7	0	2	8	0	1
Day 9	1/26/2011	spotting, count (edge v. dead)	3	0	0	5	0	2	8	0	0
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	3	0	0	3	0	2	6	1	1
Day 11	1/28/2011	spotting, count (edge v. dead)	3	0	0	2	0	1	4	0	2
Day 12	1/29/2011	transfer, (edge v. dead*), start culture	1	0	2	1	0	1	4	0	0
Day 13	1/30/2011	spotting, count (edge v. dead)	1	0	0	0	0	1	2	0	2
Day 14	1/31/2011	transfer, (edge v. dead*), start culture	1	0	0	0	0	0	2	0	0
Day 15	2/1/2011	spotting, count (edge v. dead)	0	0	1				2	0	0
Day 16	2/2/2011	transfer, (edge v. dead*), start culture	0	0	0				2	0	0
Day 17	2/3/2011	spotting, count (edge v. dead)							1	0	1
Day 18	2/4/2011	transfer, (edge v. dead*), start culture							0	0	1
Day 19	2/5/2011	spotting, count (edge v. dead)							0	0	0

		Strain: fer-1 on WT SN250	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3	1/13/2011	start cultures (E.coli)									
Day -2	1/14/2011	egg prep + spotting, start cultures									
Day -1	1/15/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	5	0	0	5	0	0	5	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	5	0	0	5	0	0	5	0	0
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	5	0	0	5	0	0	5	0	0
Day 3	1/20/2011	spotting, count (edge v. dead)	4	0	1	5	0	0	5	0	0
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	4	0	0	5	0	0	5	0	0
Day 5	1/22/2011	spotting, count (edge v. dead)	4	0	0	5	0	0	5	0	0
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	4	0	0	4	1	0	4	0	1
Day 7	1/24/2011	spotting, count (edge v. dead)	4	0	0	4	0	0	4	0	0
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	3	0	1	3	0	1	3	1	0
Day 9	1/26/2011	spotting, count (edge v. dead)	3	0	0	2	0	1	2	0	1
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	2	1	0	2	0	0	2	0	0
Day 11	1/28/2011	spotting, count (edge v. dead)	1	0	1	2	0	0	1	0	1
Day 12	1/29/2011	transfer, (edge v. dead*), start culture	1	0	0	0	0	2	1	0	0
Day 13	1/30/2011	spotting, count (edge v. dead)	1	0	0	0	0	0	1	0	0
Day 14	1/31/2011	transfer, (edge v. dead*), start culture	0	0	1				1	0	0
Day 15	2/1/2011	spotting, count (edge v. dead)	0	0	0				0	0	1
Day 16	2/2/2011	transfer, (edge v. dead*), start culture							0	0	0

		Strain: fer-1 on zcf 15 -/-	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	1/13/2011	start cultures (E.coli)									
Day -2	1/14/2011	egg prep + spotting, start cultures									
Day -1	1/15/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	14	0	6	18	0	2	20	0	0
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	9	5	0	13	1	4	13	1	6
Day 3	1/20/2011	spotting, count (edge v. dead)	9	0	0	8	0	5	10	0	3
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	9	0	0	5	0	3	9	0	1
Day 5	1/22/2011	spotting, count (edge v. dead)	9	0	0	5	0	0	4	0	5
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	8	0	1	5	0	0	1	0	3
Day 7	1/24/2011	spotting, count (edge v. dead)	8	0	0	5	0	0	1	0	0
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	5	1	2	4	0	1	1	0	0
Day 9	1/26/2011	spotting, count (edge v. dead)	4	0	1	4	0	0	1	0	0
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	3	0	1	2	0	2	1	0	0
Day 11	1/28/2011	spotting, count (edge v. dead)	2	0	1	1	0	1	1	0	0
Day 12	1/29/2011	transfer, (edge v. dead*), start culture	0	0	2	0	0	1	1	0	0
Day 13	1/30/2011	spotting, count (edge v. dead)	0	0	0	0	0	0	0	0	1
Day 14	1/31/2011	transfer, (edge v. dead*), start culture							0	0	0

		Strain: fer-1 on zcf 15 -/-	Plate A			Plate B			Plate C		
Day	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3	1/14/2011	start cultures (E.coli)									
Day -2	1/15/2011	egg prep + spotting, start cultures									
Day -1	1/16/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	5	0	0	5	0	0	5	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	5	0	0	5	0	0	5	0	0
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	5	0	0	5	0	0	5	0	0
Day 3	1/20/2011	spotting, count (edge v. dead)	5	0	0	5	0	0	5	0	0
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	3	0	2	5	0	0	5	0	0
Day 5	1/22/2011	spotting, count (edge v. dead)	3	0	0	5	0	0	4	0	1
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	1	0	2	2	0	3	2	0	2
Day 7	1/24/2011	spotting, count (edge v. dead)	1	0	0	2	0	0	2	0	0
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	1	0	0	1	1	0	2	0	0
Day 9	1/26/2011	spotting, count (edge v. dead)	1	0	0	1	0	0	2	0	0
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	1	0	0	1	0	0	1	0	1
Day 11	1/28/2011	spotting, count (edge v. dead)	0	0	1	0	0	1	1	0	0
Day 12	1/29/2011	transfer, (edge v. dead*), start culture	0	0	0	0	0	0	1	0	0
Day 13	1/30/2011	spotting, count (edge v. dead)							1	0	0
Day 14	1/31/2011	transfer, (edge v. dead*), start culture							1	0	0
Day 15	2/1/2011	spotting, count (edge v. dead)							1	0	0
Day 16	2/2/2011	transfer, (edge v. dead*), start culture							1	0	0
Day 17	2/3/2011	spotting, count (edge v. dead)							1	0	0
Day 18	2/4/2011	transfer, (edge v. dead*), start culture							1	0	0
Day 19	2/5/2011	spotting, count (edge v. dead)							0	0	1
Day 20	2/6/2011	count (edge v. dead), spot							0	0	0

		Strain: fer-1 on ORF 19.1219	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	1/14/2011	start cultures (E.coli)									
Day -2	1/15/2011	egg prep + spotting, start cultures									
Day -1	1/16/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	19	0	1	16	2	2	20	0	0
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	19	0	0	12	3	1	14	6	0
Day 3	1/20/2011	spotting, count (edge v. dead)	18	0	1	12	0	0	14	0	0
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	13	0	5	11	0	1	11	0	3
Day 5	1/22/2011	spotting, count (edge v. dead)	11	0	2	10	0	1	10	0	1
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	9	0	2	8	0	2	6	0	4
Day 7	1/24/2011	spotting, count (edge v. dead)	9	0	0	8	0	0	5	0	1
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	8	1	0	6	0	2	3	0	2
Day 9	1/26/2011	spotting, count (edge v. dead)	8	0	0	3	0	3	3	0	0
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	5	0	3	1	0	2	3	0	0
Day 11	1/28/2011	spotting, count (edge v. dead)	5	0	0	0	0	1	1	0	2
Day 12	1/29/2011	transfer, (edge v. dead*), start culture	3	0	2	0	0	0	0	0	1
Day 13	1/30/2011	spotting, count (edge v. dead)	2	0	1				0	0	0
Day 14	1/31/2011	transfer, (edge v. dead*), start culture	1	0	1						
Day 15	2/1/2011	spotting, count (edge v. dead)	1	0	0						
Day 16	2/2/2011	transfer, (edge v. dead*), start culture	0	0	1						

		Strain: fer-1 on ORF 19.1219	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	1/14/2011	start cultures (E.coli)									
Day -2	1/15/2011	egg prep + spotting, start cultures									
Day -1	1/16/2011	spotting									
Day 0	1/17/2011	transfer same # of worms, start culture	5	0	0	5	0	0	5	0	0
Day 1	1/18/2011	spotting, count (edge v. dead)	5	0	0	5	0	0	4	0	1
Day 2	1/19/2011	transfer, (edge v. dead*), start culture	4	0	1	5	0	0	4	0	0
Day 3	1/20/2011	spotting, count (edge v. dead)	4	0	0	4	0	1	4	0	0
Day 4	1/21/2011	transfer, (edge v. dead*), start culture	4	0	0	4	0	0	3	1	0
Day 5	1/22/2011	spotting, count (edge v. dead)	4	0	0	4	0	0	3	0	0
Day 6	1/23/2011	transfer, (edge v. dead*), start culture	4	0	0	3	0	1	2	0	1
Day 7	1/24/2011	spotting, count (edge v. dead)	3	0	1	2	0	1	2	0	0
Day 8	1/25/2011	transfer, (edge v. dead*), start culture	1	0	2	1	0	1	1	0	1
Day 9	1/26/2011	spotting, count (edge v. dead)	0	0	1	1	0	0	1	0	0
Day 10	1/27/2011	transfer, (edge v. dead*), start culture	0	0	0	1	0	0	0	0	1
Day 11	1/28/2011	spotting, count (edge v. dead)				0	0	1	0	0	0
Day 12	1/29/2011	transfer, (edge v. dead*), start culture				0	0	0			

		Strain: 2.5 mg/mL	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	12/2/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	12/3/2011	count (edge v. dead)	20	0	0	19	0	1	20	0	0
Day 2	12/4/2011	count (edge v. dead), start cultures	19	0	1	18	0	1	20	0	0
Day 3	12/5/2011	count (edge v. dead), spot	19	0	0	18	0	0	20	0	0
Day 4	12/6/2011	transfer (edge v. dead*)	19	0	0	18	0	0	20	0	0
Day 5	12/7/2011	count (edge v. dead)	19	0	0	15	0	3	17	0	3
Day 6	12/8/2011	count (edge v. dead) start cultures	14	0	5	15	0	0	15	0	2
Day 7	12/9/2011	count (edge v. dead), spot	14	0	0	15	0	0	12	0	3
Day 8	12/10/2011	transfer (edge v. dead)	12	0	2	11	0	4	11	0	1
Day 9	12/11/2011	count (edge v. dead), start cultures	10	0	2	10	0	1	10	0	1
Day 10	12/12/2011	count (edge v. dead), spot	6	0	4	5	0	5	8	0	2
Day 11	12/13/2011	transfer (edge v. dead)	6	0	0	4	0	1	7	0	1
Day 12	12/14/2011	count (edge v. dead), start cultures	1	0	5	2	0	2	2	0	5
Day 13	12/15/2011	count (edge v. dead), spot	1	0	0	2	0	0	1	0	1
Day 14	12/16/2011	transfer (edge v. dead)	0	0	1	2	0	0	0	0	1
Day 15	12/17/2011	count (edge v. dead)	0	0	0	1	0	1	0	0	0
Day 16	12/18/2011	count (edge v. dead) start cultures	0	0	0	0	0	1	0	0	0

		Strain: 5 mg/mL	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	12/2/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	12/3/2011	count (edge v. dead)	19	0	1	19	0	1	20	0	0
Day 2	12/4/2011	count (edge v. dead), start cultures	18	0	1	18	0	1	19	0	1
Day 3	12/5/2011	count (edge v. dead), spot	18	0	0	18	0	0	19	0	0
Day 4	12/6/2011	transfer (edge v. dead*)	18	0	0	18	0	0	19	0	0
Day 5	12/7/2011	count (edge v. dead)	16	0	2	16	0	2	16	0	3
Day 6	12/8/2011	count (edge v. dead) start cultures	16	0	0	15	0	1	16	0	0
Day 7	12/9/2011	count (edge v. dead), spot	15	0	1	12	1	2	16	0	0
Day 8	12/10/2011	transfer (edge v. dead)	13	0	2	12	0	0	16	0	0
Day 9	12/11/2011	count (edge v. dead), start cultures	13	0	0	10	0	2	12	0	4
Day 10	12/12/2011	count (edge v. dead), spot	5	0	8	8	0	2	12	0	0
Day 11	12/13/2011	transfer (edge v. dead)	5	0	0	5	0	3	7	0	5
Day 12	12/14/2011	count (edge v. dead), start cultures	0	0	5	2	0	3	4	0	3
Day 13	12/15/2011	count (edge v. dead), spot	0	0	0	2	0	0	1	0	3
Day 14	12/16/2011	transfer (edge v. dead)	0	0	0	1	0	1	1	0	0
Day 15	12/17/2011	count (edge v. dead)	0	0	0	0	0	1	0	0	1

		Strain: 10 mg/mL	Plate A			Plate B			Plate C		
	Date	Task	Alive	Edge	Dead	Alive	Edge	Dead	Alive	Edge	Dead
Day -3		start cultures (E.coli)									
Day -2		egg prep + spotting, start cultures									
Day -1		spotting									
Day 0	12/2/2011	transfer same # (20) of worms,	20	0	0	20	0	0	20	0	0
Day 1	12/3/2011	count (edge v. dead)	19	0	1	19	0	1	19	0	1
Day 2	12/4/2011	count (edge v. dead), start cultures	18	1	0	18	0	1	18	0	1
Day 3	12/5/2011	count (edge v. dead), spot	18	0	0	18	0	0	18	0	0
Day 4	12/6/2011	transfer (edge v. dead*)	18	0	0	18	0	0	18	0	0
Day 5	12/7/2011	count (edge v. dead)	18	0	0	16	0	2	17	0	1
Day 6	12/8/2011	count (edge v. dead) start cultures	18	0	0	16	0	0	16	0	1
Day 7	12/9/2011	count (edge v. dead), spot	18	0	0	14	0	2	16	0	0
Day 8	12/10/2011	transfer (edge v. dead)	11	0	7	11	0	3	16	0	0
Day 9	12/11/2011	count (edge v. dead), start cultures	7	0	4	11	0	0	15	0	1
Day 10	12/12/2011	count (edge v. dead), spot	3	0	4	5	0	6	10	0	5
Day 11	12/13/2011	transfer (edge v. dead)	3	0	0	3	0	2	5	0	5
Day 12	12/14/2011	count (edge v. dead), start cultures	0	0	3	2	0	1	3	0	2
Day 13	12/15/2011	count (edge v. dead), spot	0	0	0	1	0	1	1	0	2
Day 14	12/16/2011	transfer (edge v. dead)	0	0	0	0	0	1	1	0	0
Day 15	12/17/2011	count (edge v. dead)	0	0	0	0	0	0	0	0	1

		Strain: Control (0µM fluconazole)	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	3/15/2012	start cultures (E.coli)									
Day -2	3/16/2012	egg prep + spotting, start cultures									
Day -1	3/17/2012	spotting									
Day 0	3/18/2012	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	3/19/2012	spotting, count (edge v. dead)	16	4	0	19	1	0	19	1	0
Day 2	3/20/2012	transfer, (edge v. dead*), start culture	15	0	1	19	0	0	13	3	3
Day 3	3/21/2012	spotting, count (edge v. dead)	13	0	2	17	1	1	11	0	2
Day 4	3/22/2012	transfer, (edge v. dead*), start culture	9	0	4	15	0	2	7	1	3
Day 5	3/23/2012	spotting, count (edge v. dead)	6	0	3	11	2	2	5	0	2
Day 6	3/24/2012	transfer, (edge v. dead*), start culture	2	0	4	10	0	1	5	0	0
Day 7	3/25/2012	spotting, count (edge v. dead)	2	0	0	8	0	2	2	0	3
Day 8	3/26/2012	transfer, (edge v. dead*), start culture	1	0	0	5	0	3	2	0	0
Day 9	3/27/2012	spotting, count (edge v. dead)	1	0	0	4	0	1	1	0	1
Day 10	3/28/2012	transfer, (edge v. dead*), start culture	0	0	1	3	0	1	1	0	0
Day 11	3/29/2012	spotting, count (edge v. dead)				1	0	2	1	0	0
Day 12	3/30/2012	transfer, (edge v. dead*), start culture				0	0	1	0	0	1

		Strain: 50μM fluconazole	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	3/15/2012	start cultures (E.coli)									
Day -2	3/16/2012	egg prep + spotting, start cultures									
Day -1	3/17/2012	spotting									
Day 0	3/18/2012	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	3/19/2012	spotting, count (edge v. dead)	18	2	0	18	2	0	18	2	0
Day 2	3/20/2012	transfer, (edge v. dead*), start culture	16	1	1	18	0	0	17	1	0
Day 3	3/21/2012	spotting, count (edge v. dead)	13	3	0	13	3	2	17	0	0
Day 4	3/22/2012	transfer, (edge v. dead*), start culture	13	0	0	12	0	1	17	0	0
Day 5	3/23/2012	spotting, count (edge v. dead)	13	0	0	10	0	2	17	0	0
Day 6	3/24/2012	transfer, (edge v. dead*), start culture	10	0	3	9	0	1	16	0	1
Day 7	3/25/2012	spotting, count (edge v. dead)	8	0	2	8	0	1	12	0	4
Day 8	3/26/2012	transfer, (edge v. dead*), start culture	7	0	1	7	0	1	12	0	0
Day 9	3/27/2012	spotting, count (edge v. dead)	7	0	0	5	0	2	10	0	2
Day 10	3/28/2012	transfer, (edge v. dead*), start culture	4	0	3	0	0	5	8	0	2
Day 11	3/29/2012	spotting, count (edge v. dead)	2	0	2				2	0	6
Day 12	3/30/2012	transfer, (edge v. dead*), start culture	1	0	1				0	0	2
Day 13	3/31/2012	spotting, count (edge v. dead)	0	0	1						

		Strain: 100μM fluconazole	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	3/15/2012	start cultures (E.coli)									
Day -2	3/16/2012	egg prep + spotting, start cultures									
Day -1	3/17/2012	spotting									
Day 0	3/18/2012	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	3/19/2012	spotting, count (edge v. dead)	16	4	0	18	2	0	19	1	0
Day 2	3/20/2012	transfer, (edge v. dead*), start culture	16	0	0	15	0	3	16	2	1
Day 3	3/21/2012	spotting, count (edge v. dead)	15	1	0	14	1	0	16	0	0
Day 4	3/22/2012	transfer, (edge v. dead*), start culture	15	0	0	9	0	5	14	0	2
Day 5	3/23/2012	spotting, count (edge v. dead)	15	0	0	9	0	0	14	0	0
Day 6	3/24/2012	transfer, (edge v. dead*), start culture	15	0	0	8	0	1	10	0	4
Day 7	3/25/2012	spotting, count (edge v. dead)	14	0	1	4	0	4	8	0	2
Day 8	3/26/2012	transfer, (edge v. dead*), start culture	12	0	2	3	0	1	6	0	2
Day 9	3/27/2012	spotting, count (edge v. dead)	7	0	5	2	0	1	3	0	3
Day 10	3/28/2012	transfer, (edge v. dead*), start culture	3	0	4	1	0	1	1	0	2
Day 11	3/29/2012	spotting, count (edge v. dead)	2	0	1	1	0	0	0	0	1
Day 12	3/30/2012	transfer, (edge v. dead*), start culture	0	0	2	0	0	1			

		Strain: 150μM fluconazole	Plate A			Plate B			Plate C		
Day	**Date**	**Task**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**	**Alive**	**Edge**	**Dead**
Day -3	3/15/2012	start cultures (E.coli)									
Day -2	3/16/2012	egg prep + spotting, start cultures									
Day -1	3/17/2012	spotting									
Day 0	3/18/2012	transfer same # of worms, start culture	20	0	0	20	0	0	20	0	0
Day 1	3/19/2012	spotting, count (edge v. dead)	18	2	0	18	2	0	16	4	0
Day 2	3/20/2012	transfer, (edge v. dead*), start culture	18	0	0	18	0	0	16	0	0
Day 3	3/21/2012	spotting, count (edge v. dead)	18	0	0	18	0	0	16	0	0
Day 4	3/22/2012	transfer, (edge v. dead*), start culture	18	0	0	13	0	5	13	0	3
Day 5	3/23/2012	spotting, count (edge v. dead)	17	1	0	10	0	3	13	0	0
Day 6	3/24/2012	transfer, (edge v. dead*), start culture	15	2	0	6	0	4	11	0	2
Day 7	3/25/2012	spotting, count (edge v. dead)	15	0	0	6	0	0	6	0	5
Day 8	3/26/2012	transfer, (edge v. dead*), start culture	13	0	2	5	0	1	4	0	2
Day 9	3/27/2012	spotting, count (edge v. dead)	10	0	3	5	0	0	3	0	1
Day 10	3/28/2012	transfer, (edge v. dead*), start culture	4	0	6	2	0	3	1	0	2
Day 11	3/29/2012	spotting, count (edge v. dead)	2	0	2	0	0	2	0	0	1
Day 12	3/30/2012	transfer, (edge v. dead*), start culture	1	0	0						
Day 13	3/31/2012	spotting, count (edge v. dead)	0	0	1						

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