Analyzing Microbial Interactions in the Gut Microbiome

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A Major Qualifying Project Report submitted to the Faculty of Worcester Polytechnic Institute in partial fulfillment of the Degree of Bachelor of Science

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Abstract

Probiotics are used to maintain a healthy gut microbiome. Although *Candida albicans* is part of our natural microbiota, dysbiosis has been associated with Crohn's disease and Irritable Bowel Syndrome. Our project evaluates the interactions between *C. albicans* and the probiotic *Bacillus subtilis* on the health and longevity of *Caenorhabditis elegans*, a model host. Our findings will identify genes and pathways that regulate microbial interactions in the gut and inform antifungal drug development.

Acknowledgements

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Introduction

A microbiome is a collection of microorganisms working together in various environments throughout the body. A complex microbiome in the human body is the gut microbiome, which is composed of bacteria, protozoa, viruses, and fungi. In this microbiome, the bacterial cells present outnumber the native human cells by a factor of 10 (Bull & Plummer, 2014). This abundance of diversity in the gut microbiome is important as it allows for many functions to be completed, but it also creates ample opportunity for the healthy balance in the biome to be disrupted. Imbalances in the gut microbiome can result in ailments such as Irritable Bowel Syndrome, Inflammatory Bowel Disease, systemic metabolic diseases, allergies, and more (Bull & Plummer, 2014). Imbalances can occur due to lifestyle choices, such as what we eat or the medicine we take, depletion of immune responses, or even anxiety and stress.

Microbiotics

It is estimated that there are $5x10^{30}$ bacteria in the world (University of Georgia, 1998). These bacteria can be found in the environment, in your water, and even all throughout your body. There are multiple types of bacteria and they all perform various functions. Some bacteria cause disease when they enter your body, while others are beneficial and aid in performing vital functions for life. These good bacteria are called probiotics. Probiotics are live, nonpathogenic microorganisms that are either administered or found naturally in the body (Williams, 2010). The most common examples of bacteria as probiotics are *Bacillus, Lactobacillus,* and *Bifidobacterium* (Fijan, 2014), and they are found naturally occurring in the human body in the mouth, urinary tract, skin, and gut. They aid in functions such as digestion, overall oral hygiene, and reduction of allergies by inhibiting the growth of pathogens. While it has become more popular for people to take probiotic supplements, when in balance, the body is equipped with all the probiotics it needs to support a healthy life. It is essential to have these good microbes in combination with the bad microbes, as they create a balance in the human microbiome.

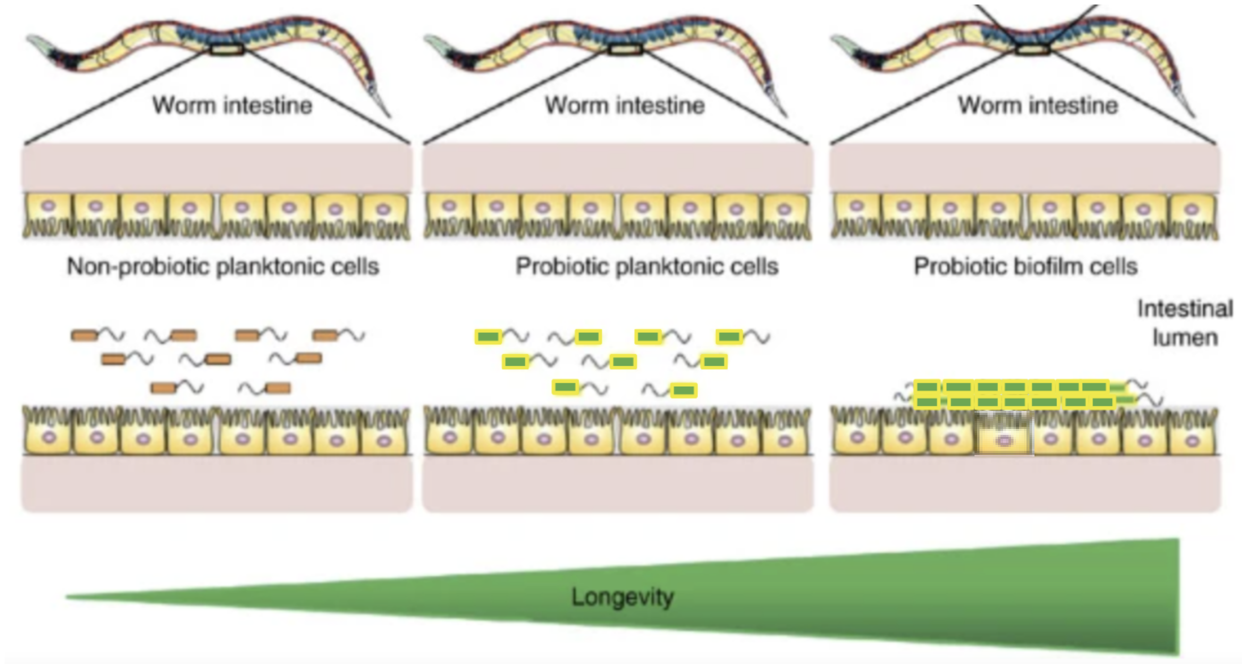

Figure 1: The presence of biofilms in the *C. elegans* intestines
(Adapted from Donato, V., Ayala, F., Cogliati, S. *et al.*)

When the microbiome is out of balance, probiotics can be used to rebalance the environment and help reduce levels of harmful microorganisms. The mechanisms by which probiotics function includes production of bioactive metabolites, competing for resources with pathogens, and producing biofilms, which are cooperative communities of adherent cells within a self-produced matrix (Ayala et al, 2017). A potential probiotic used in treatment of the gut microbiome is *Bacillus,* which has been found to naturally colonize in healthy gut microbiomes (Ilinskaya et al, 2017). *Bacillus* are rod-shaped, gram positive bacteria that produce spores and have been shown to have antimicrobial, antioxidative, and immune-modulatory effects in the gut microbiome. In particular, *Bacillus subtilis* has proved to be an important keystone species. Once its spores are consumed, they germinate in the intestine and the active form of *B. subtilis* creates a biofilm in the host intestine (Figure 1). The biofilm cells produce beneficial and anti-aging molecules in the host tissues, demonstrating the positive effects that *B. subtilis* provides. This probiotic behavior of *B. subtilis* has shown particular promise in the gut of *Caenorhabditis elegans*, creating a biofilm and increasing the longevity of the species. (Ayala et al, 2017).

Probiotics aim to target a multitude of disrupting organisms. One of these main disrupting organisms in the gut are opportunistic pathogens, which are pathogens that reside in the human body and typically do not cause disease unless under certain conditions, such as low immune response. While antibiotics, antivirals, and antifungals can be used to treat opportunistic pathogens, probiotics are arising as potential treatments (Chew et al, 2015). Antibiotics have been found to alter the taxonomic, genomic, and functional capacity of the human gut microbiota, leading to diseases, antibiotic resistant bacteria, and other persisting complications. Conversely, probiotics are thought to be positive alternatives that prevent and treat infections while maintaining a healthy microbiota balance (Modi, Collins, & Relman, 2014). Research has shown that probiotics can cause adverse effects to biofilm spread by interfering with the adherence of yeast cells through secretion of antimicrobial compounds (Chew et al, 2015). One common opportunistic pathogen found in the human body is the fungus *Candida albicans*. *C. albicans* is the most abundant fungus in humans, residing primarily in the gastrointestinal tract as part of the normal microbiome. It is a harmless commensal organism in most individuals, but has pathogenic potential if overgrown, which can occur under conditions such as stress or illness. *C. albicans* have a complex effect on human hosts because they can exist in multiple morphological forms (Figure 1). The two most common of these forms are yeast and hyphae. Yeast cells are prolific during the commensal stage while hyphae are of the invasive form. A balanced microbiome helps to control the amount of *C. albicans* in the hyphae form (Tyc et al, 2014). However, when the microbiome becomes imbalanced or the immune system is compromised, *C. albicans* population can increase rapidly, leading to infection. These infections range from cutaneous to systemic.

Investigating Candida albicans Infections

Candida albicans colonization of the gut has been linked to several diseases including Crohn's Disease, Irritable Bowel Syndrome, and pulmonary inflammatory responses. A virulence factor of *C. albicans* is its ability to thrive as a biofilm, especially in the gut. Consequently, the destruction of biofilms is a major target for antifungal therapies. However, *C. albicans* biofilms are resistant to conventional antifungal treatments (Nobile & Johnson, 2015). Thus, it is important that new therapies become available. Studies investigating the use of probiotics have been ongoing as they have shown promise as a more novel antifungal treatment in not only inhibiting the formation of pathogenic biofilms but forming healthy biofilms to rebalance the microbiome. In 2015, one study explored the interaction of different probiotic lactobacilli strains and *Candida glabrata* and found that two strains completely inhibited *C. glabrata* biofilms and downregulated the related genes EPA6 and YAK1. The results suggested that the inhibition of the biofilms was accomplished by interfering with the adherence of the yeast cells (Chew et al, 2015).

To investigate scientific inquiries such as the relationship between fungi and probiotics, the nematode *Caenorhabditis elegans* is often used. *C. elegans* is an organism approximately 1mm in length and is an exceptional model organism for a variety of reasons. *C. elegans* have many of the organ systems present in more complex organisms, including a digestive system, nervous system, muscular system, and reproductive system. In addition, they display complex behaviors such as eating, reproducing, and decision making while moving. These behaviors and systems, combined with their small stature, make them prime model hosts to use in the lab (Apfeld & Alper, 2018). Additionally, the organism is anatomically simple, so its development has been described in immense detail. *C. elegans* are either males or hermaphrodites, allowing them to reproduce quickly, frequently, and without need of mating. The gut of *C. elegans* is also anatomically similar to mammals as both have polarized epithelial cells with apical microvilli (Pukkila-Worley & Ausubel, 2012). In addition, the *C. elegans* intestine not only absorbs nutrients, but detoxifies metabolites and toxins, similar to the liver in humans (Jiang & Wang, 2018). Studies have shown that roughly 30–60% of genes in *C. elegans* have orthologs or strong homologs in mammals, allowing for conclusions to be drawn relating findings in *C. elegans* to humans and other large mammals (Alberts et al, 2002). For example, research conducted to identify a novel innate immunity regulatory pathway conserved in *C. elegans* and mammals revealed that the TFEB gene, the mammalian ortholog of the HLH-30 transcription factor in *C. elegans*, is a key innate immunity regulator in mammals (Apfeld & Alper, 2018). Furthermore, the exact lineage of every cell of *C. elegans* and its complete genome sequence has been mapped, so much can be predicted regarding the fates of the organism's cells (Alberts et al, 2002). Finally, *C. elegans* are easy to maintain using NGM plates and *Escherichia coli* as their food source, and accelerated reproduction allows for stocks to be kept easily.

The research into probiotics as a potential antimicrobial treatment is promising. While probiotics have yet to gain FDA approval for specific diseases, they have demonstrated positive results in the prevention and treatment of a plethora of diseases in humans. In addition, probiotics prove beneficial in other situations, such as improving crop microbiota and soil regeneration. Imbalances between microbiota in the human microbiome can cause potentially detrimental diseases, but can be managed with the correct treatment. With the gut microbiome being such a complex environment, maintenance of this balance is even more vital. *C. albicans,* the most abundant fungi in the gastrointestinal system, accounts for over half of all cases of candidiasis, which is the leading cause of mycosis-associated mortality in the United States (Pfaller & Diekema, 2007). Investigating the relationship between *C. albicans* and *B. subtilis*, a promising probiotic with antimicrobial properties found in the gut microbiome, is crucial for finding an effective treatment to avoid systemic infections. Utilizing the ability of *B. subtilis* to colonize healthy biofilms has potential to inhibit the adhesion of, or balance out, *C. albicans* pathogenic biofilms. The scientific goal of this project is to evaluate the potential differential interactions between *C. albicans* mutants and *B. subtilis* through their effects on *C. elegans* host lifespans. This will be accomplished through a series of survival assays to determine which *C. albicans* mutants show differential interactions with *B. subtilis* to alter the lifespan of *C. elegans*. From this data we can gain a better understanding of the genes and pathways in the fungal pathogen, *C. albicans*, and their contribution to the probiotic interaction with *B. subtilis*, in the context of *C. elegans* as a live animal model host.

Materials and Methods

Strains, Media, and Growth Conditions

The Suzanne Noble *Candida albicans* deletion library was used for this project, provided by the Fungal Genetics Stock Center. For wild type and mutant *C. albicans,* wild type *Bacillus subtilis*, and *Escherichia coli* strain OP50, media and growth conditions used are described below. The wildtype used was SN250, which has the genotype *his1Δ/his1Δ, leu2Δ::C dubliniensis HIS1/leu2Δ::C.maltosa LEU2, arg4Δ/arg4Δ, URA3/ura3Δ::imm*^{434}*, IRO1/iro1Δ::imm*^{434} (Noble & Johnson, 2005). SN250 was used as a wild type control because markers that were used to delete the genes in the deletion library, Arg and Leu, were replaced in SN250.

Candida albicans

C. albicans were grown on Yeast-Peptone-Dextrose (YPD) agar plates at 30°C. Liquid cultures of the wild type and mutant strains of *C. albicans* were inoculated and grown in YPD overnight at 30°C in a roller-drum (Calderone & Cihlar, 2009).

Bacillus subtilis

Liquid cultures of *B. subtilis* were inoculated and grown in Tryptic Soy Broth (TSB) overnight at 30°C in a roller drum. The liquid culture was then streaked and grown on Luria Broth (LB) agar plates at 30°C overnight. Liquid cultures of wildtype *B. subtilis* were inoculated and grown in Tryptic Soy Broth (TSB) overnight at 30°C in a roller drum (Calderone & Cihlar, 2009).

Escherichia coli

E. coli OP50 was grown in LB overnight at 30°C and stored at 4°C (Sambrook, Fritsch, & Maniatis, 1989).

C. elegans maintenance and Egg Preparation

C. elegans were maintained on Nematode Growth Medium (NGM) agar plates seeded with 200µL of *E. coli* OP50. Eggs were prepared from 30 worms in the L4 stage and grown at 20°C. By day 3, there were approximately 3600 eggs per NGM plate. Worms and eggs were washed off the plates with up to 10mL of M9 buffer, or until all the eggs were off the plate. The worm, egg, and buffer mixture was then centrifuged (1.5 minutes, 3,500 rpm). The supernatant was aspirated leaving about 1mL, and the worms were resuspended in a 1:4 bleach dilution containing 1mL of 0.25 M sodium hydroxide and 4mL of 10% bleach. This suspension was mixed gently by inversion for no more than 2 minutes until the majority of adult worms had disintegrated and only eggs were left, which could be seen under a dissection microscope. The suspension was then centrifuged again (1.5 minutes, 2,500 rpm). The supernatant was aspirated leaving about 1mL, and the egg pellet was resuspended in 10mL of M9 buffer. The egg suspension was centrifuged again (1.5 minutes, 2,500 rpm). After the supernatant was aspirated, the pellet was resuspended in 200 µL of M9 buffer. Each plate was seeded with 20µL of the final egg suspension until each plate yielded approximately 35 eggs or until the 200 µL of solution was used up (Figure 2).

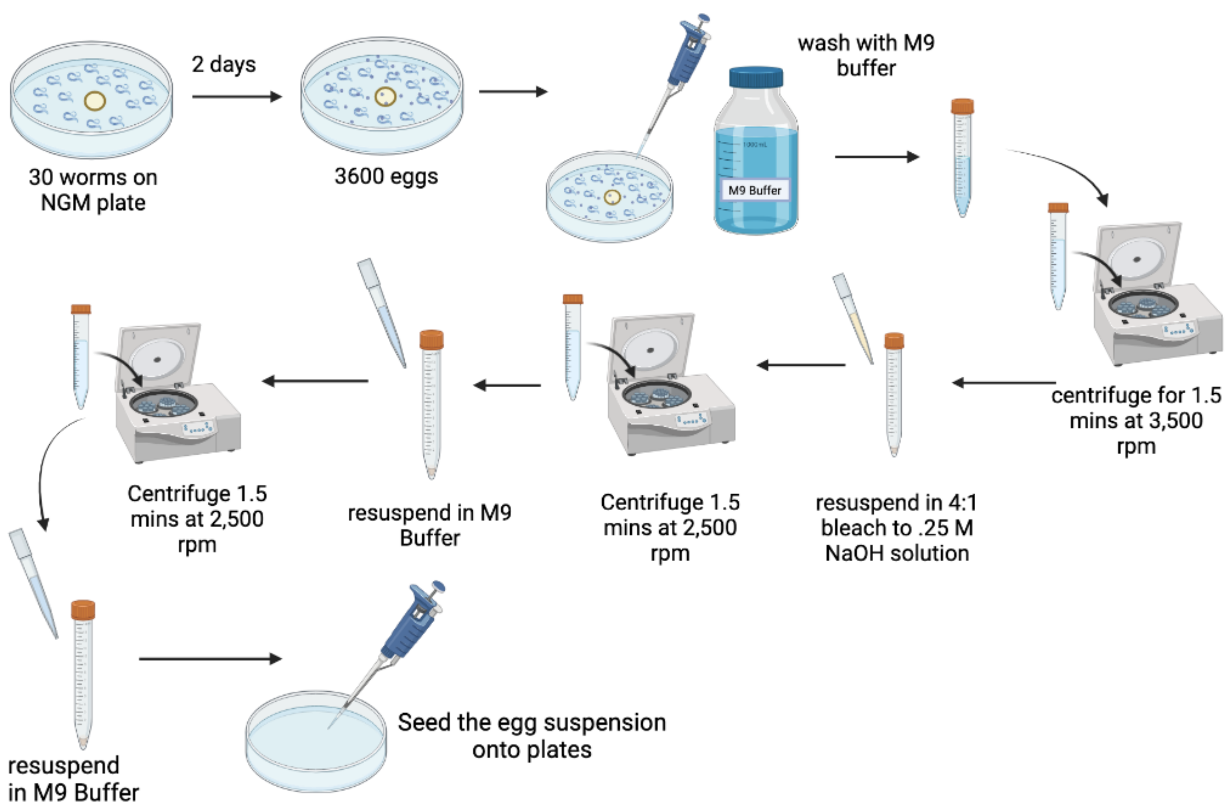

Figure 2: The steps of performing egg preparation

C. elegans Survival Assay

To track mortality of infected *C. elegans,* a serial infection assay was conducted as follows. 30 adult worms were grown on an NGM agar plate seeded with 120 µL of *E. coli* OP50. Eggs were harvested. Approximately 35 eggs were placed onto plates seeded with 120 µL of either wild type *B. subtilis* or OP50 (control). Worms were grown to adulthood (48 hours), at which point all worms were transferred onto fresh plates seeded with 120 µL of mutant *C. albicans* every other day until death. The control assay was performed with wildtype *C. albicans* (Figure 3).

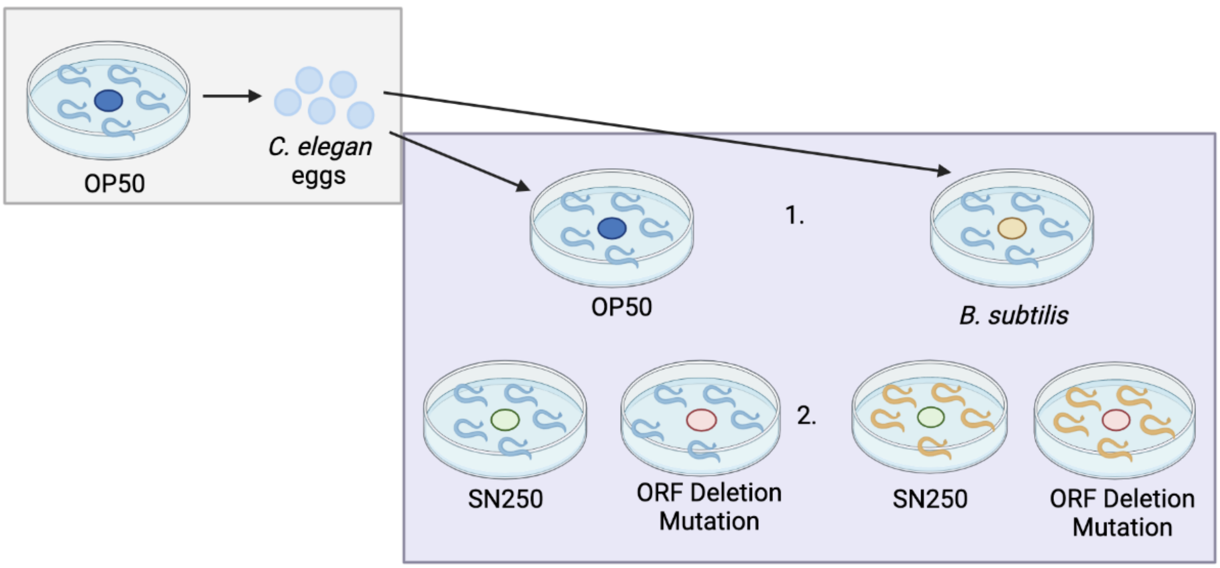

Figure 3: The outline of the survival assay setup

To ensure that the same number of *C. albicans* and *B. subtilis* cells were used for each assay, cultures were standardized to an Optical Density (A_{600}) of 1.0. 500 µL of each incubated liquid culture were centrifuged on a benchtop microcentrifuge (10 minutes, 3,200 rpm). The culture supernatant was aspirated, and the pellet was resuspended in 500µL of sterile deionized water and centrifuged again (5 minutes, 3,200 rpm). The culture supernatant was aspirated, and the pellet was resuspended in 500µL of deionized water. Absorbance at 600 nm (A_{600}) was measured for at 1:10 dilution of the culture using a spectrophotometer that had previously been blanked with water alone. For yeast, this standardized to 1.89 x 10^7 cells, and for bacteria this standardized to about 8 x 10^8 cells. Finally, 120 µL of each culture aliquot was seeded onto 4 NGM agar plates (2 for mutant *C. albicans* and 2 for wildtype *C. albicans)* and air dried on the benchtop overnight. The survival assay began with dispensing the *C. elegans* egg suspension onto an NGM plate seeded with OP50 and onto another plate seeded with *B. subtilis*. *C. elegans* were grown on these plates for approximately two days. After plating the *C. elegans* egg suspension, approximately 30 worms pre-exposed to *B. subtilis* and 30 worms without pre-exposure were transferred to separate NGM plates seeded with mutant *C. albicans* and wildtype *C. albicans*. There were a total of 4 plates (Figure 4):

1. WT *C. albicans* + *C. elegans* pre-treated with OP50
2. WT *C. albicans* + *C. elegans* pre-treated with *B. Subtilis*
3. Mutant *C. albicans* + *C. elegans* pre-treated with OP50
4. Mutant *C. albicans* + *C. elegans* pre-treated with *B. Subtilis*

The next day, worms alive, dead, and missing were counted and documented. The next day, the living worms were transferred to new NGM plates seeded with mutant and wildtype *C. albicans*. This was repeated until all the worms were dead. All the mutant experiments were conducted twice in parallel with the wildtype as a control. Refer to Appendix I for a step-by-step procedure for conducting a survival assay.

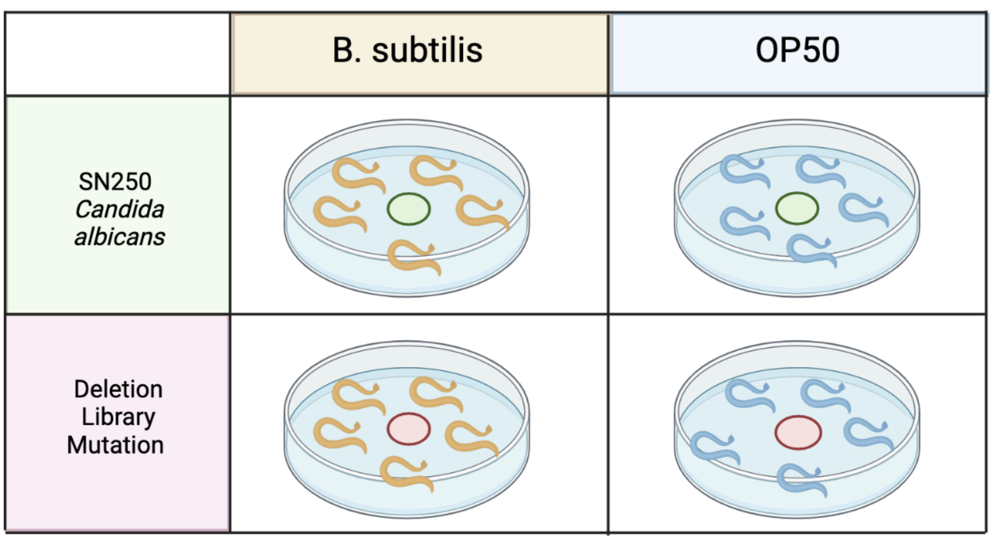

Figure 4: The 4 plate conditions the survival assay contains

Analysis and maintenance of laboratory notes

Starting each assay, the strain of *C. albicans*, the date, and the number of worms plated were recorded. Each day until all the worms were dead, the number of alive, dead, and missing worms in each assay was recorded. *C. elegans* were considered dead if they did not move freely or respond to physical stimuli, such as touch by the transferring pick. If worms were accidentally killed on transfer or could not be located on the plate, they were considered missing and were not considered when testing for statistical significance. In these cases, the worms were considered to be censored from the study because the event experienced was neither alive nor dead. The time that has elapsed to that point of the study is known, but it cannot be determined how much time will elapse before these worms are dead. Thus, the data is entered to indicate these worms were censored (Motulsky, 2021). The mutant survival assays were then analyzed and compared with and without pre-exposure to *B. subtilis*, and then also compared to the wildtype *C. albicans* survival curve with and without pre-exposure to *B. subtilis* to test for different survival patterns and determine a potential mechanism for probiotic action of *B. subtilis*.

All data was analyzed using GraphPad Prism and tested for statistical significance using LogRank and Gehan-Breslow-Wilcoxon tests. Both tests were used as analysis tools to compare the rates of death for assays with and without pre-exposure to *B. subtilis* and to determine if there was a statistically significant difference between the two groups. In the LogRank test, each death in a survival assay is weighted the same, so it was used to determine if the ratio of deaths per day was equal at each time point for both groups. The Gehan-Breslow-Wilcoxon test places greater weight on deaths that occur early-on in the survival assay and was used to determine which group has a higher risk of death (GraphPad Software, Inc., n.d.). With the combination of these two tests, the difference in survival rates of each group and the statistical significance was determined.

When creating a new data set in GraphPad, the following options were used: "survival," "enter or import data into a new table," and "enter elapsed time into a new table". The different treatment groups were put under the group labels, which were SN250 + *B. subtilis*, SN250 - *B. subtilis*, mutant + *B. subtilis*, and mutant - *B. subtilis.* The number of days was put in the X-column. For each day, the number of alive and missing worms were put in the data set. The number 1 was used for dead worms, and the number 0 was used for missing worms. For every dead or missing worm in each group, that amount of 1 or 0 were put in their columns. Once all the data was entered, the relationships between every treatment group was analyzed, for a total of

6 comparisons. The WT groups used black lines, and the mutant groups used red lines. Any group treated with *B. subtilis* used a solid line, and untreated groups used a dashed line. Under the results tab, the LogRank and Gehan-Breslow significance was analyzed. For each comparison, the Gehan-Breslow significance was recorded; if it was a yes, the p-value was recorded. See Appendix II for a step-by-step procedure of a survival assay GraphPad analysis. The four comparisons completed were:

I. SN250 - *B. subtilis* vs. mutant - *B. subtilis*
II. SN250 + *B. subtilis* vs. mutant + *B. subtilis*
III. Mutant + *B. subtilis* vs. mutant - *B. subtilis*
IV. SN250 + *B. subtilis* vs. SN250 - *B. subtilis* (Control)

Results

Deletion Library Mutation	Significance Trial 1		Significance Trial 2	
ORF19.1002Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1049Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1162Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1171Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1239Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1345Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1365Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1449Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +
	III	μ + vs. μ -	III	μ + vs. μ -
ORF19.1502Δ	I	WT - vs. μ -	I	WT - vs. μ -
	II	WT + vs. μ +	II	WT + vs. μ +

	Ⅲ	μ + vs. μ -	Ⅲ	μ + vs. μ -
ORF19.1586Δ	Ⅰ	WT - vs. μ -	Ⅰ	WT - vs. μ -
	Ⅱ	WT + vs. μ +	Ⅱ	WT + vs. μ +
	Ⅲ	μ + vs. μ -	Ⅲ	μ + vs. μ -

Legend 1: Comparisons with statistically significant p-values for the comparisons. (Ⅰ) SN250 - *B. subtilis* vs Mutant - *B. subtilis,* (Ⅱ) SN250 + *B. subtilis* vs Mutant + *B. subtilis,* (Ⅲ) Mutant + *B. subtilis* vs. Mutant - *B. subtilis*, (Ⅳ) SN250 - *B. subtilis* vs. SN250 + *B. subtilis* are highlighted in red. The Ⅳ comparison not included as it was the control. The wild type SN250 is written as wildtype and the deletion library mutant is written as μ. Strains treated with *B. subtilis* are written with a "+" and strains not treated with *B. subtilis* are treated with a "-".

Out of 700 mutants in the Suzanne Noble gene knockout library, we have screened a total of 10. Out of the ones we screened, only 6 showed a significant difference in virulence compared to the SN250 in both trials. This means that while *C. elegans* infected with wild type *Candida albicans* normally died at around 10 days, *C. elegans* infected with these mutant *Candida albicans* lived for a time statistically significantly different from that, either longer or shorter. Mutations are found at about a 10% rate. The mutants in the Suzanne Noble library are preselected because of their suspected abnormal phenotype that is expected to have an effect on virulence of *Candida albicans*. This explains why 10 of the screened gene knockouts showed a statistically significant difference when only 1/10 genes in the entire genome are expected to have a significant mutation. Each mutant was assayed twice for a total of two trials per deletion library mutation. For each mutant n = 1 and 30 worms were studied on each experimental plate. Two trials were run for each mutation to ensure consistent results and increase confidence in the conclusions drawn for each deletion mutation. In all plots below, wild type *C. albicans* treated with *B. subtilis* is represented with a solid black line, wildtype *C. albicans* without *B. subtilis* treatment is represented with a dotted black line, mutant *C. albicans* with *B. subtilis* treatment is represented with a solid red line, and mutant *C. albicans* without *B. subtilis* treatment is represented with a dotted red line. From this, 4 comparisons were drawn (Last figure in methods). The same color coding from that figure is used in tables depicting lifespan of each condition throughout the results. Green represents wildtype *C. albicans*, red represents mutant *C. albicans*, yellow represents treatment with *B. subtilis*, and blue represents no *B. subtilis* treatment.

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1002Δ*.

Trial 1

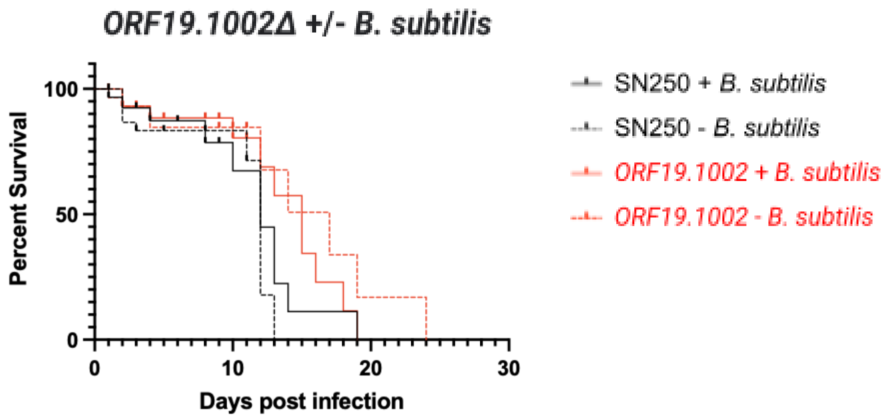

Figure 5: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1002Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1002Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1002Δ* knockout *C. albicans* mutant.

Table 1: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1002Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1002 Δ + B. sub.*	*ORF19.1002 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1002 Δ + B. sub.*	*ORF19.1002 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		NS	NS	-		NS	NS	-
SN250 - *B. sub.*	NS		-	NS	NS		-	0.0437
ORF19.1002Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1002Δ - B. sub.	-	NS	NS		-	0.0437	NS	

Table 2: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1002Δ*.

	B. subtilis	OP50
SN250 *C. albicans*	19	13
ORF19.1002Δ *C. albicans*	19	24

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1002Δ* - *B. subtilis*: Deletion of *ORF19.1002* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1002Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1002Δ* knockout mutant. The treated control lived longer than the untreated control, but the untreated *ORF19.1002Δ* lived 5 days longer than the treated *ORF19.1002Δ* which does not follow the expected pattern modeled by the black curves (Fig. 5). There was no statistically significant p-value for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0437 for the LogRank test when comparing the two groups (Table 1). This suggests that, in comparison to the control, the deletion of *ORF19.1002Δ* affects the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1002Δ* + *B. subtilis*: Deletion of *ORF19.1002* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1002Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1002Δ* knockout mutant. The treated control lived longer than the untreated control group, but the untreated *ORF19.1002Δ* lived 5 days longer than the treated *ORF19.1002Δ*, which does not follow the expected pattern modeled by the black curves (Fig. 5). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 1). This suggests that, in comparison to the control, the deletion of *ORF19.1002Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1002Δ* + *B. subtilis* vs *ORF19.1002Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1002Δ + *B. subtilis* (solid, red) was the treated *ORF19.1002Δ* knockout mutant. *ORF19.1002Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1002Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1002Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group. *ORF19.1002Δ - B. subtilis* survived 5 days longer than *ORF19.1002Δ* + *B. subtilis* (Fig. 5). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 1). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1002Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 6 days longer than SN250 - *B. subtilis* (Fig. 5). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 1). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

Trial 2

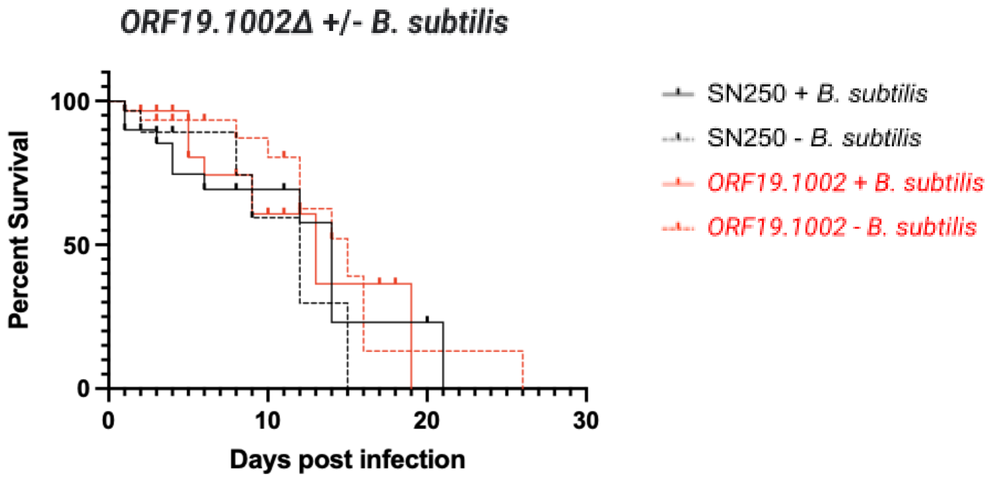

Figure 6: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1002Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1002Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1002Δ* knockout *C. albicans* mutant.

Table 3: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1002Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1002Δ + B. sub.*	*ORF19.1002Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1002Δ + B. sub.*	*ORF19.1002Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		NS	NS	-		NS	NS	-
SN250 - *B. sub.*	NS		-	NS	NS		-	NS
ORF19.1002Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1002Δ - B. sub.	-	NS	NS		-	NS	NS	

Table 4: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1002Δ*

	B. subtilis	OP50
SN250 *C. albicans*	21	15
ORF19.1002Δ C. albicans	19	26

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1002Δ - B. subtilis*: Deletion of *ORF19.1002* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1002Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1002Δ* knockout mutant. The treated control lived longer than the untreated control, but the untreated *ORF19.1002Δ* lived 7 days longer than the treated *ORF19.1002Δ* which does not follow the expected pattern modeled by the black curves (Fig. 6). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 3). This suggests that, in comparison to the control, the deletion of *ORF19.1002Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1002Δ + B. subtilis*: Deletion of *ORF19.1002* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1002Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1002Δ* knockout mutant. The treated control lived longer than the untreated control group, but the untreated *ORF19.1002Δ* lived 7 days longer than the treated *ORF19.1002Δ*, which does not follow the expected pattern modeled by the black curves (Fig. 6). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 3). This suggests that, in comparison to the control, the deletion of *ORF19.1002Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1002Δ + B. subtilis* vs *ORF19.1002Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1002Δ + *B. subtilis* (solid, red) was the treated *ORF19.1002Δ* knockout mutant. *ORF19.1002Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1002Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1002Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group. *ORF19.1002Δ - B. subtilis* survived 7 days longer than *ORF19.1002Δ + B. subtilis* (Fig. 6). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups

(Table 3). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1002Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 6 days longer than SN250 - *B. subtilis* (Fig. 6). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 3). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

Genetic Profile:

ORF19.1002 is a protein of unknown function and has a Hap43 repressed gene. *ORF19.1002* has an unknown molecular function, biological processes, and cellular components. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1049Δ*

Trial 1

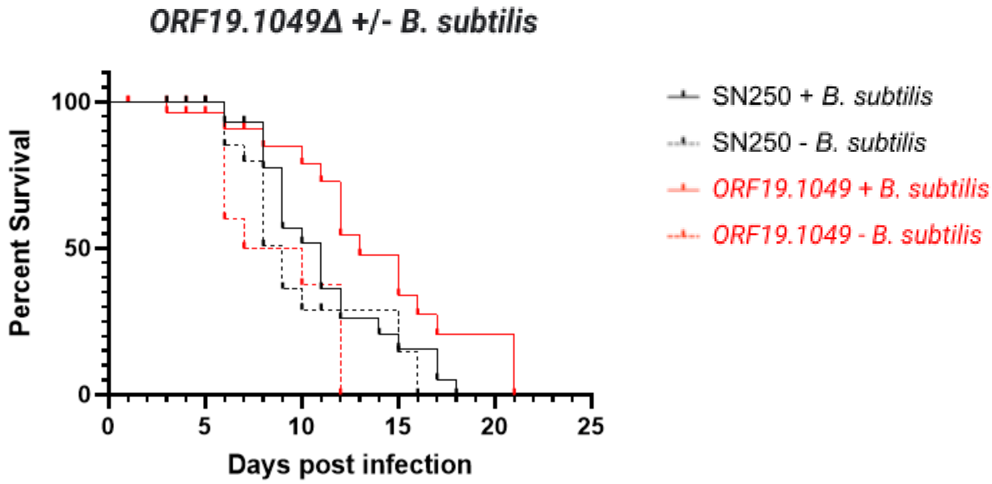

Figure 7: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1049Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1049Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1049Δ* knockout *C. albicans* mutant.

Table 5: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1049Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1049Δ + B. sub.*	*ORF19.1049Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1049Δ + B. sub.*	*ORF19.1049Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		NS	NS	-		NS	NS	-
SN250 - *B. sub.*	NS		-	NS	NS		-	NS
ORF19.1049Δ + B. sub.	NS	-		NS	NS	-		0.0139
ORF19.1049Δ - B. sub.	-	NS	NS		-	NS	0.0139	

Table 6: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1049Δ*

	B. subtilis	OP50
SN250 *C. albicans*	18	16
ORF19.1002Δ *C. albicans*	21	12

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1049Δ - B. subtilis*: Deletion of *ORF19.1049* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1049Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1049Δ* knockout mutant. Both untreated groups had shorter survival than the treated group, but the untreated *ORF19.1049Δ* knockout mutant lived 4 days less than the untreated SN250 wildtype (Fig. 7). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 5). This suggests that, in comparison to the control, the deletion of *ORF19.1049Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1049Δ + B. subtilis*: Deletion of *ORF19.1049* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1049Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1049Δ* knockout mutant. Both treated groups lived longer than the untreated groups, but the treated *ORF19.1049Δ* knockout mutant lived 3 days longer than the treated SN250 wildtype group (Fig. 7). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 5). This suggests that, in comparison to the control, the deletion of *ORF19.1049Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1049Δ + B. subtilis* vs *ORF19.1049Δ - B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

ORF19.1049Δ + *B. subtilis* (solid, red) was the treated *ORF19.1049Δ* knockout mutant. *ORF19.1049Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1049Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1049Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group. *ORF19.1049Δ* + *B. subtilis* survived 7 days longer than *ORF19.1049Δ* - *B. subtilis* (Fig. 7). There was no statistically significant p-value for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0139 for the LogRank test when comparing the two groups (Table 5). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1049Δ* knockout mutant.

(IV) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 2 days longer than SN250 - *B. subtilis* (Fig. 7). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 5). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

Trial 2

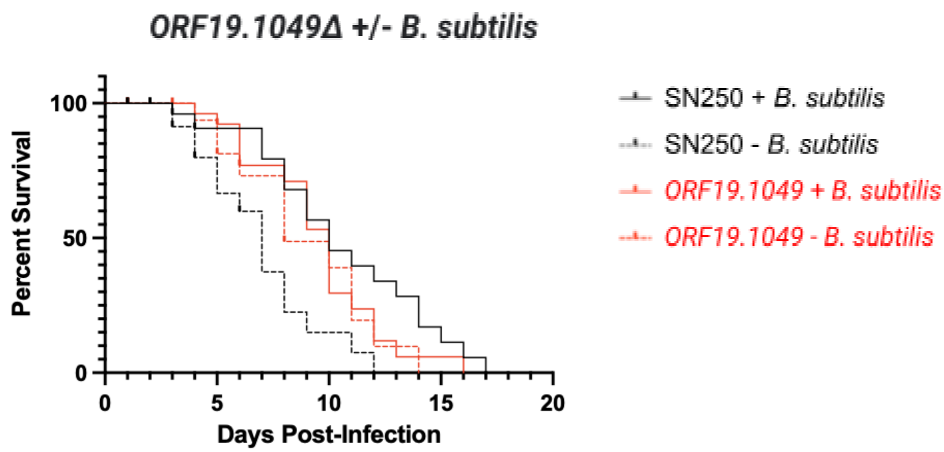

Figure 8: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1049Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1049Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1049Δ* knockout *C. albicans* mutant.

Table 7: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1049Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1049 Δ + B. sub.*	*ORF19.1049 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1049 Δ + B. sub.*	*ORF19.1049 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0111	0.5556	-		0.00026	0.2830	-
SN250 - *B. sub.*	0.0111		-	NS	0.00026		-	NS
ORF19.1049Δ + B. sub.	0.5556	-		NS	0.2830	-		0.0139
ORF19.1049 Δ - B. sub.	-	NS	NS		-	NS	0.0139	

Table 8: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1049Δ*

	B. subtilis	OP50
SN250 *C. albicans*	17	12
ORF19.1002Δ *C. albicans*	16	14

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1049Δ - B. subtilis*: Deletion of *ORF19.1049* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1049Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1049Δ* knockout mutant. Both untreated groups had shorter survival than the treated group, but the untreated *ORF19.1049Δ* knockout mutant lived 2 days longer than the untreated SN250 wildtype (Fig. 8). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 7). This suggests that, in comparison to the control, the deletion of *ORF19.1049Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1049Δ + B. subtilis*: Deletion of *ORF19.1049* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1049Δ + B. subtilis* (solid, red) was the treated *ORF19.1049Δ* knockout mutant. Both treated groups lived longer than the untreated groups, but the treated *ORF19.1049* knockout mutant lived 1 day less than the treated SN250 wildtype group (Fig. 8). There was a statistically significant p-value of 0.5556 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.2830 for the LogRank test when comparing the two groups (Table 7). This suggests that, in comparison to the control, the deletion of *ORF19.1049Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1049Δ + B. subtilis* vs *ORF19.1049Δ - B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

ORF19.1049Δ + B. subtilis (solid, red) was the treated *ORF19.1049Δ* knockout mutant. *ORF19.1049Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1049Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1049Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group. *ORF19.1049Δ + B. subtilis* survived 7 days longer than *ORF19.1049Δ - B. subtilis* (Fig. 8). There was no statistically significant p-value for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0139 for the LogRank test when comparing the two groups (Table 7). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1049Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 5 days longer than SN250 - *B. subtilis* (Fig. 8). There was a statistically significant p-value of 0.0111 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.00026 for the LogRank test when comparing the two groups (Table 7). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1049 has a predicted NUDIX hydrolase domain and is Hap43 induced. *ORF19.1049* has the molecular function of hydrolase activity. Its biological processes include GPI anchor biosynthetic processes and cytoplasm to vacuole transport via the Cvt pathway. The *ORF19.1049Δ* cellular components are unknown. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1162Δ*

<u>Trial 1</u>

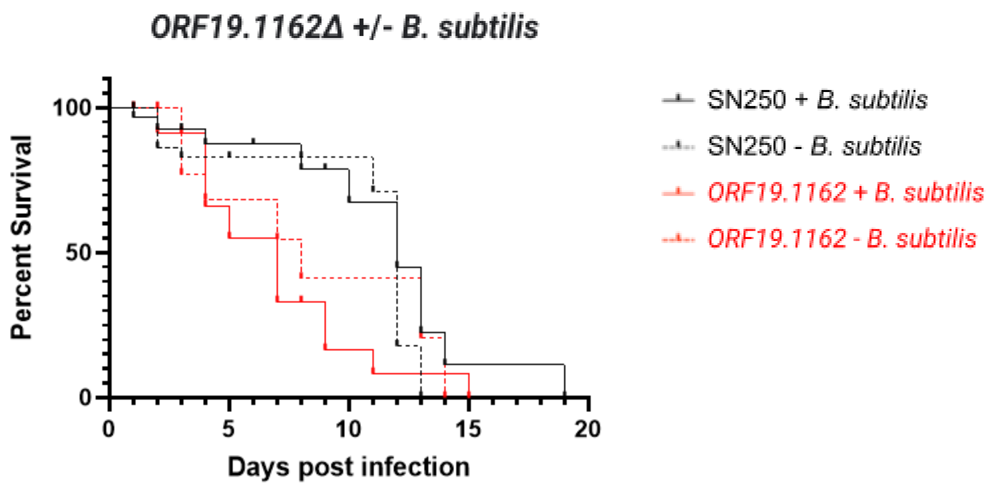

Figure 9: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1162Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1162Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1162Δ* knockout *C. albicans* mutant.

Table 9: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1162Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1162Δ* + *B. sub.*	*ORF19.1162Δ* - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1162Δ* + *B. sub.*	*ORF19.1162Δ* - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		NS	0.0412	-		NS	0.0099	-
SN250 - *B. sub.*	NS		-	NS	NS		-	NS
ORF19.1162Δ + *B. sub.*	0.0412	-		NS	0.0099	-		NS
ORF19.1162Δ - *B. sub.*	-	NS	NS		-	NS	NS	

Table 10: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1162Δ*

	B. subtilis	OP50
SN250 *C. albicans*	19	13
ORF19.1002Δ *C. albicans*	15	14

(I) SN250 - *B. subtilis* vs *ORF19.1162Δ - B. subtilis*: Deletion of *ORF19.1162* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1162Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1162Δ* knockout mutant. Both untreated groups had shorter survival than the treated group, but the untreated *ORF19.1162Δ* knockout mutant lived 1 day longer than the untreated SN250 wildtype (Fig. 9). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 9). This suggests that, in comparison to the control, the deletion of *ORF19.1162Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(II) SN250 + *B. subtilis* vs *ORF19.1162Δ + B. subtilis*: Deletion of *ORF19.1162* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1162Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1162Δ* knockout mutant. Both treated groups lived longer than the untreated groups, but the treated *ORF19.1162Δ* knockout mutant lived 1 day less than the treated SN250 wildtype group (Fig. 9). There was a statistically significant p-value of 0.0412 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0099 for the LogRank test when comparing the two groups (Table 9). This suggests that, in comparison to the control, the deletion of *ORF19.1162Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(III) *ORF19.1162Δ + B. subtilis* vs *ORF19.1162Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1162Δ + *B. subtilis* (solid, red) was the treated *ORF19.1162Δ* knockout mutant. *ORF19.1162Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1162Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1162Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group. *ORF19.1162Δ* + *B. subtilis* survived 7 days longer than *ORF19.1162Δ* - *B. subtilis* (Fig. 9). There was no statistically significant p-value for the Gehan-Breslow or LogRank test when comparing the two groups (Table 9). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1162Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 6 days longer than SN250 - *B. subtilis* (Fig. 9). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table 9). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

<u>Trial 2</u>

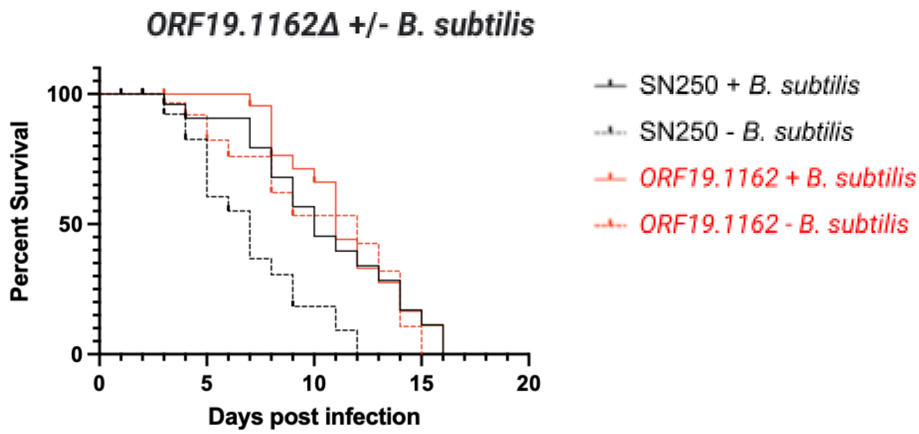

Figure 10: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1162Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1162Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1162Δ* knockout *C. albicans* mutant.

Table 11: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1162Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1162 Δ + B. sub.*	*ORF19.1162 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1162 Δ + B. sub.*	*ORF19.1162 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0131	NS	-		0.0041	NS	-
SN250 - *B. sub.*	0.0131		-	0.0406	0.0041		-	0.0068
ORF19.1162Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1162Δ - B. sub.	-	0.0406	NS		-	0.0068	NS	

Table 12: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1162Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	12
ORF19.1002Δ C. albicans	16	15

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1162Δ - B. subtilis*: Deletion of *ORF19.1162* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1162Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1162Δ* knockout mutant. Both untreated groups had shorter survival than the treated group, but the untreated *ORF19.1162Δ* knockout mutant lived 3 days longer than the untreated SN250 wildtype (Fig. 10). There was a statistically significant p-value of 0.0406 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0068 for the LogRank test when comparing the two groups (Table. 11). This suggests that, in comparison to the control, the deletion of *ORF19.1162Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1162Δ + B. subtilis*: Deletion of *ORF19.1162* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1162Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1162Δ* knockout mutant. Both treated groups lived longer than the untreated groups and the treated *ORF19.1162Δ* knockout mutant lived the same amount of time as the treated SN250 wildtype group (Fig. 10). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 11). This suggests that, in comparison to the control, the deletion of *ORF19.1162Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1162Δ + B. subtilis* vs *ORF19.1162Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1162Δ + *B. subtilis* (solid, red) was the treated *ORF19.1162Δ* knockout mutant. *ORF19.1162Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1162Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1162Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group. *ORF19.1162Δ* + *B. subtilis* survived 3 days longer than *ORF19.1162Δ* - *B. subtilis* (Fig. 10). There was no statistically significant p-value for the Gehan-Breslow or LogRank test when comparing the two groups

(Table. 11). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1162Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 4 days longer than SN250 - *B. subtilis* (Fig. 10). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 11). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

Genetic Profile:

ORF19.1162 is a protein of unknown function and its transcript is upregulated by benomyl treatment. *ORF19.1162* has an unknown molecular function, biological processes, and cellular components. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1171Δ*

Trial 1

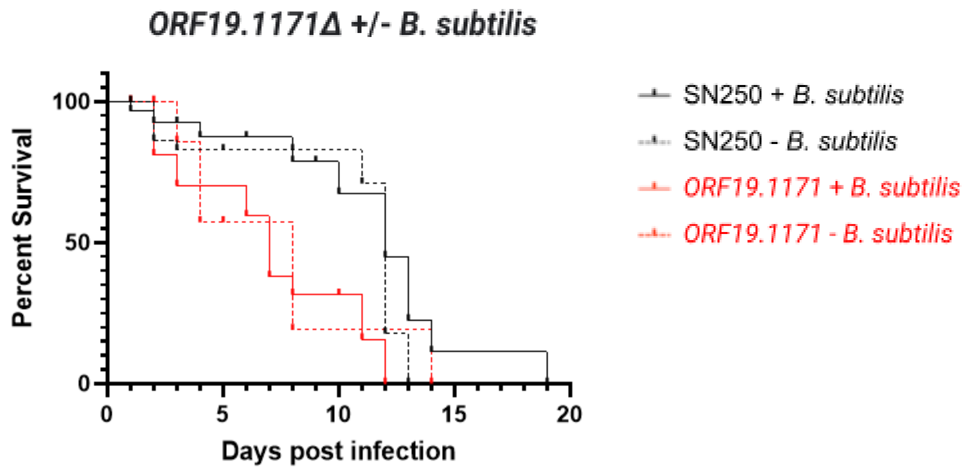

Figure 11: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1171Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1171Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1171Δ* knockout *C. albicans* mutant.

Table 13: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1171Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1171Δ + B. sub.*	*ORF19.1171Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1171Δ + B. sub.*	*ORF19.1171Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		NS	0.0301	-		NS	0.0041	-
SN250 - *B. sub.*	NS		-	NS	NS		-	NS
ORF19.1171Δ + B. sub.	0.0301	-		NS	0.0041	-		NS
ORF19.1171Δ - B. sub.	-	NS	NS		-	NS	NS	

Table 14: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1171Δ*

	B. subtilis	OP50
SN250 *C. albicans*	19	13
ORF19.1002Δ *C. albicans*	12	14

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1171Δ - B. subtilis*: Deletion of *ORF19.1171* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1171Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1171Δ* knockout mutant. The SN250 treated group survived 6 days longer than the treated group, but the untreated *ORF19.1171Δ* group survived 2 days longer than the treated group (Fig. 11). There were no statistically significant p-values for the Gehan-Breslow and LogRank tests when comparing the two groups (Table. 13). This suggests that, in comparison to the control, the deletion of *ORF19.1171Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1171Δ + B. subtilis*: Deletion of *ORF19.1171* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1171Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1171Δ* knockout mutant. The treated SN250 group lived 7 days longer than the treated *ORF19.1171Δ* knockout mutant group (Fig 11). There was a statistically significant p-value of 0.0301 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0041 for the LogRank test when comparing the two groups (Table. 13). This suggests that, in comparison to the control, the deletion of *ORF19.1171Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1171Δ + B. subtilis* vs *ORF19.1171Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1171Δ + *B. subtilis* (solid, red) was the treated *ORF19.1171Δ* knockout mutant. *ORF19.1171Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1171Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1171Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group. *ORF19.1171Δ* - *B. subtilis* survived 2 days longer than *ORF19.1171Δ* + *B. subtilis* (Fig. 11). There was no statistically significant p-value for the Gehan-Breslow or LogRank test when comparing the two groups (Table. 13). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1171Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 4 days longer than SN250 - *B. subtilis* (Fig. X). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 13). This suggests that pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control. This control data is not consistent with the hypothesis that pre-exposure to *B. subtilis* contributes to survival of the wild type SN250 infected *C. elegans* model. This could have resulted from factors such as an older *C. elegans* population used in the assay that would result in early death regardless of the *B. subtilis* treatment or too many censored worms to calculate significance.

Trial 2

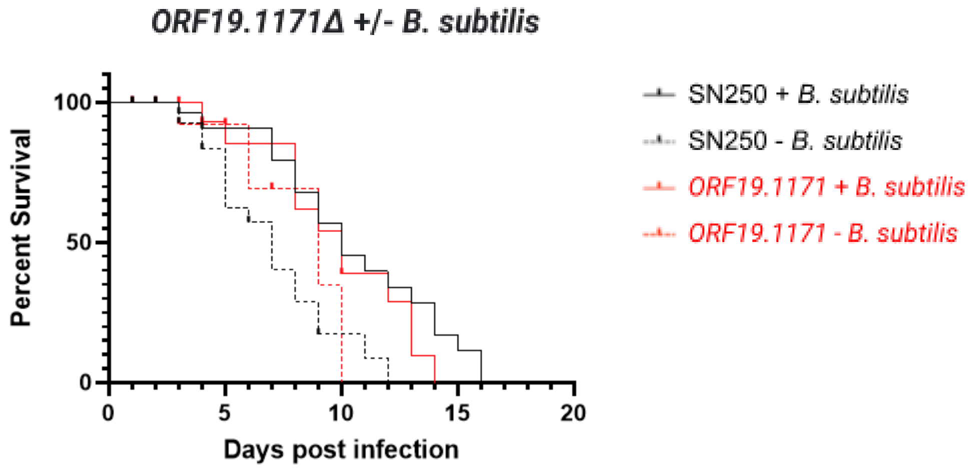

Figure 12: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1171Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1171Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1171Δ* knockout *C. albicans* mutant.

Table 15: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1171Δ.* Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1171 Δ + B. sub.*	*ORF19.1171 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1171 Δ + B. sub.*	*ORF19.1171 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0124	NS	-		0.0034	NS	-
SN250 - *B. sub.*	0.0124		-	NS	0.0034		-	NS
ORF19.1171Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1171Δ - B. sub.	-	NS	NS		-	NS	NS	

Table 16: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1171Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	12
ORF19.1002Δ C. albicans	14	10

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1171Δ - B. subtilis*: Deletion of *ORF19.1171* does not affect in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1171Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1171Δ* knockout mutant. The SN250 treated group survived 2 days longer than the untreated group, but the treated *ORF19.1171Δ* group survived 4 days longer than the untreated group (Fig. 12). There were no statistically significant p-values for the Gehan-Breslow and LogRank tests when comparing the two groups (Table. 15). This suggests that, in comparison to the control, the deletion of *ORF19.1171Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1171Δ + B. subtilis*: Deletion of *ORF19.1171* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1171Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1171Δ* knockout mutant. The treated SN250 group lived 2 days longer than the treated *ORF19.1171Δ* knockout mutant group (Fig. 12). There were no statistically significant p-values for the Gehan-Breslow or the LogRank test when comparing the two groups (Table. 15). This suggests that, in comparison to the control, the deletion of *ORF19.1171Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1171Δ + B. subtilis* vs *ORF19.1171Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1171Δ + *B. subtilis* (solid, red) was the treated *ORF19.1171Δ* knockout mutant. *ORF19.1171Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1171Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1171Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group. *ORF19.1171Δ* + *B. subtilis* survived 4 days longer than *ORF19.1171Δ* - *B. subtilis* (Fig. 12). There was no statistically significant p-value for the Gehan-Breslow or LogRank test when comparing the two groups (Table. 15). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1171Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the B. subtilis treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 4 days longer than SN250 - *B. subtilis* (Fig. 12). There was a statistically significant p-value of 0.0124 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0034 for the LogRank test when comparing the two groups (Table. 15). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1171 is an ortholog of *C. dubliniensis CD36* : Cd36_10780, *C. parapsilosis CDC317* : CPAR2_208080, *C. auris B8441* : B9J08_003841 and *Candida tenuis NRRL Y-1498* : CANTEDRAFT_120956. *ORF19.1171* has an unknown molecular function, biological processes, and cellular components. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a decreased rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1239Δ*

Trial 1

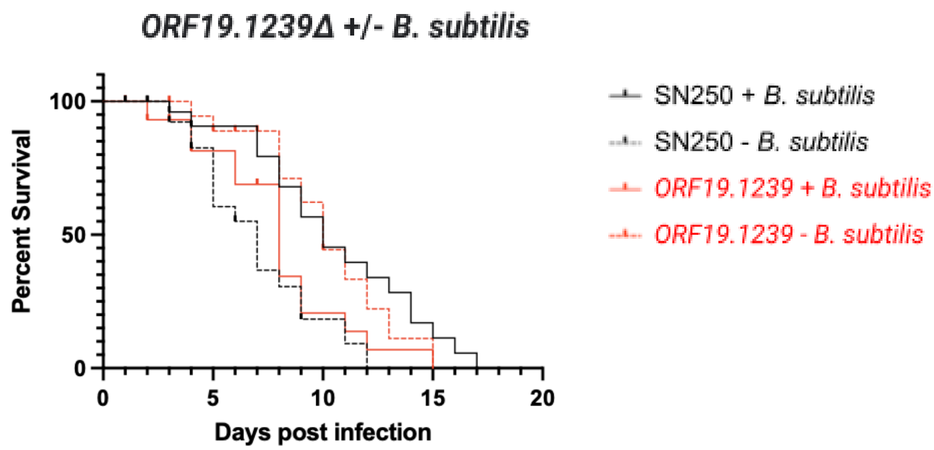

Figure 13: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1239Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1239Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1239Δ* knockout *C. albicans* mutant.

Table 17: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1239Δ.* Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1239 Δ + B. sub.*	*ORF19.1239 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1239 Δ + B. sub.*	*ORF19.1239 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0131	NS	-		0.0041	0.0484	-
SN250 - *B. sub.*	0.0131		-	0.0056	0.0041		-	0.0052
ORF19.1239Δ + B. sub.	NS	-		0.0379	0.0484	-		NS
ORF19.1239Δ - B. sub.	-	0.0056	0.0379		-	0.0052	NS	

Table 18: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1239Δ*

	B. subtilis	OP50
SN250 *C. albicans*	17	12
ORF19.1002Δ *C. albicans*	15	15

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1239Δ - B. subtilis*: Deletion of *ORF19.1239* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1239Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1239Δ* knockout mutant. The SN250 treated group survived 5 days longer than the untreated group, but the treated *ORF19.1239Δ* group survived the same amount of time as the untreated group (Fig. 13). There was a statistically significant p-value of 0.0056 for the Gehan-Breslow test when comparing the two groups. There was a statistically significant p-value of 0.0052 for the LogRank test when comparing the two groups (Table. 17). This suggests that, in comparison to the control, the deletion of *ORF19.1239Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1239Δ + B. subtilis*: Deletion of *ORF19.1239* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1239Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1239Δ* knockout mutant. The treated SN250 group lived 2 days longer than the treated *ORF19.1239Δ* knockout mutant group (Fig. 13). There were no statistically significant p-values for the Gehan-Breslow test, but there was a statistically significant p-value of 0.0484 for the LogRank test when comparing the two groups (Table. 17). This suggests that, in comparison to the control, the deletion of *ORF19.1239Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1239Δ* + *B. subtilis* vs *ORF19.1239Δ* - *B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1239Δ + *B. subtilis* (solid, red) was the treated *ORF19.1239Δ* knockout mutant. *ORF19.1239Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1239Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1239Δ* knockout mutants is not similar to the control, as the treated group lived the same amount of time as the untreated group (Fig. 13). There was a statistically significant p-value of 0.0379 for the Gehan-Breslow test when comparing the two groups. There was no statistically significant p-value for the LogRank test when comparing the two groups (Table. 17). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1239Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 5 days longer than SN250 - *B. subtilis* (Fig. 13). There was a statistically significant p-value of 0.0131 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0041 for the LogRank test when comparing the two groups (Table. 17). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Trial 2

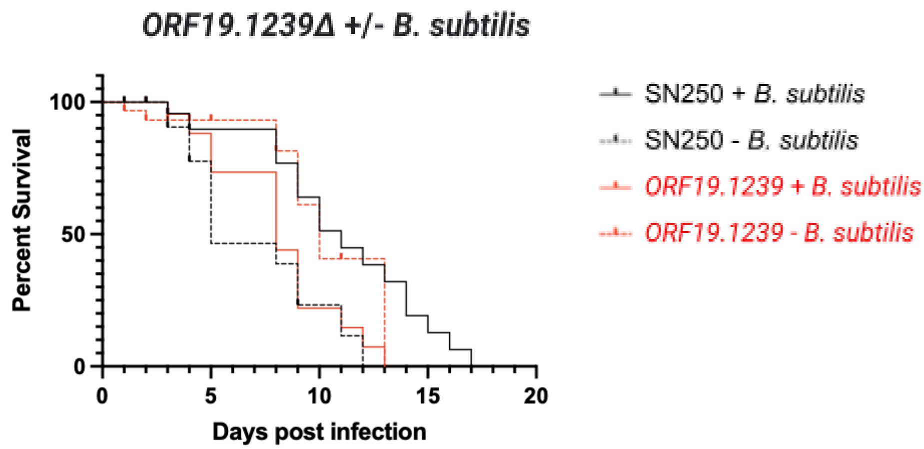

Figure 14: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1239Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1239Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1239Δ* knockout *C. albicans* mutant.

Table 19: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1239Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1239Δ + B. sub.*	*ORF19.1239Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1239Δ + B. sub.*	*ORF19.1239Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		0.0081	0.0137	-
SN250 - *B. sub.*	0.0245		-	NS	0.0081		-	0.0289
ORF19.1239Δ + *B. sub.*	NS	-		NS	0.0137	-		NS
ORF19.1239Δ - *B. sub.*	-	NS	NS		-	0.0289	NS	

Table 20: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1239Δ*

	B. subtilis	OP50
SN250 *C. albicans*	17	12
ORF19.1002Δ C. albicans	13	13

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1239Δ - B. subtilis*: Deletion of *ORF19.1239* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1239Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1239Δ* knockout mutant. The SN250 treated group survived 5 days longer than the untreated group, but the treated *ORF19.1239Δ* group survived the same amount of time as the untreated group (Fig. 14). There was no statistically significant p-value for the Gehan-Breslow test when comparing the two groups. There was a statistically significant p-value of 0.0289 for the LogRank test when comparing the two groups (Table. 19). This suggests that, in comparison to the control, the deletion of *ORF19.1239Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1239Δ + B. subtilis*: Deletion of *ORF19.1239* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1239Δ + B. subtilis* (solid, red) was the treated *ORF19.1239Δ* knockout mutant. The treated SN250 group lived 4 days longer than the treated *ORF19.1239Δ* knockout mutant group (Fig. 14). There were no statistically significant p-values for the Gehan-Breslow test, but there was a statistically significant p-value of 0.0137 for the LogRank test when comparing the two groups (Table 19). This suggests that, in comparison to the control, the deletion of *ORF19.1239Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1239Δ + B. subtilis* vs *ORF19.1239Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1239Δ + B. subtilis (solid, red) was the treated *ORF19.1239Δ* knockout mutant. *ORF19.1239Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1239Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1239Δ* knockout mutants is not similar to the control, as the treated group lived the same amount of time as the untreated group (Fig. 14). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table 19). This suggests that, in comparison to the control,

pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1239Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 5 days longer than SN250 - *B. subtilis* (Fig. 14). There was a statistically significant p-value of 0.0245 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0081 for the LogRank test when comparing the two groups (Table 19). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1239 is a secreted protein, and when the protein is exogenously expressed it is a substrate for Kex2 processing in vitro. It is fluconazole-regulated and spider biofilm induced. *ORF19.1239* has an unknown molecular function, biological processes, and cellular components. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1345Δ*

Trial 1

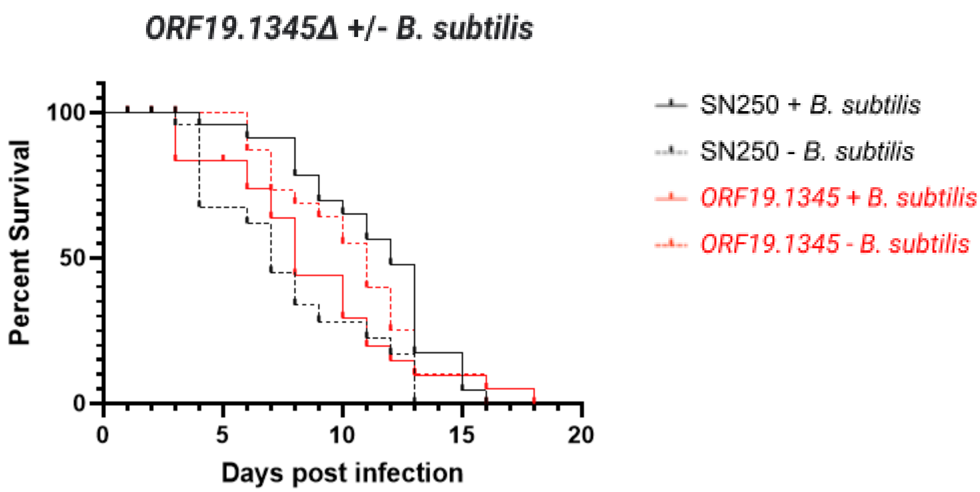

Figure 15: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1345Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1345Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1345Δ* knockout *C. albicans* mutant.

Table 21: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1345Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1345Δ + B. sub.*	*ORF19.1345Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1345Δ + B. sub.*	*ORF19.1345Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0011	0.0083	-		0.0022	NS	-
SN250 - *B. sub.*	0.0011		-	0.0172	0.0022		-	NS
ORF19.1345Δ + *B. sub.*	0.0083	-		NS	NS	-		NS
ORF19.1345Δ - *B. sub.*	-	0.0172	NS		-	NS	NS	

Table 22: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1345Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	13
ORF19.1002Δ *C. albicans*	18	16

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1345Δ - B. subtilis*: Deletion of *ORF19.1345* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1239Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1345Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the treated *ORF19.1345Δ* group survived 2 days longer than the untreated group (Fig. 15). There was a statistically significant p-value of 0.0172 for the Gehan-Breslow test when comparing the two groups. There was no statistically significant p-value for the LogRank test when comparing the two groups (Table 21). This suggests that, in comparison to the control, the deletion of *ORF19.1345Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1345Δ + B. subtilis*: Deletion of *ORF19.1345* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1345Δ + B. subtilis* (solid, red) was the treated *ORF19.1345Δ* knockout mutant. The treated SN250 group lived 2 days less than the treated *ORF19.1345Δ* knockout mutant group (Fig. 15). There was a statistically significant p-value of 0.0083 for the Gehan-Breslow test, but there was no statistically significant p-value for the LogRank test when comparing the two groups (Table 21). This suggests that, in comparison to the control, the deletion of *ORF19.1345Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1345Δ* + *B. subtilis* vs *ORF19.1345Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1345Δ + *B. subtilis* (solid, red) was the treated *ORF19.1345Δ* knockout mutant. *ORF19.1345Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1345Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1345Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 15). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table 21). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1345Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 15). There was a statistically significant p-value of 0.0011 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0022 for the LogRank test when comparing the two groups (Table 21). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

<u>Trial 2</u>

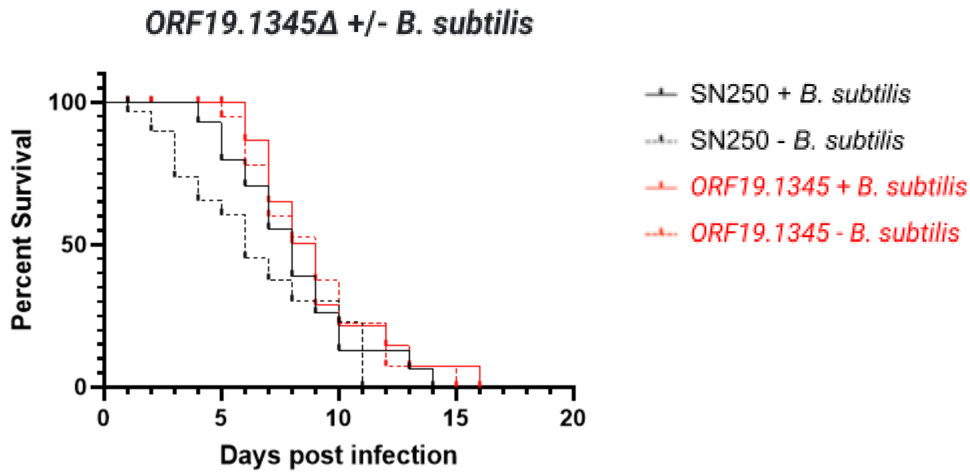

Figure 16: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1345Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1345Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1345Δ* knockout *C. albicans* mutant.

Table 23: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1345Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1345 Δ + B. sub.*	*ORF19.1345 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1345 Δ + B. sub.*	*ORF19.1345 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		NS	NS	-
SN250 - *B. sub.*	0.0245		-	0.0082	NS		-	0.0311
ORF19.1345Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1345Δ - B. sub.	-	0.0082	NS		-	0.0311	NS	

Table 24: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1345Δ*

	B. subtilis	OP50
SN250 *C. albicans*	14	11
ORF19.1002Δ C. albicans	16	15

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1345Δ - B. subtilis*: Deletion of *ORF19.1345* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1239Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1345Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the treated *ORF19.1345Δ* group survived 1 day longer than the untreated group (Fig. 16). There was a statistically significant p-value of 0.0082 for the Gehan-Breslow test when comparing the two groups. There was a statistically significant p-value of 0.0311 for the LogRank test when comparing the two groups (Table. 23). This suggests that, in comparison to the control, the deletion of *ORF19.1345Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1345Δ + B. subtilis*: Deletion of *ORF19.1345* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1345Δ + B. subtilis* (solid, red) was the treated *ORF19.1345Δ* knockout mutant. The treated SN250 group lived 2 days less than the treated *ORF19.1345Δ* knockout mutant group (Fig. 16). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 23). This suggests that, in comparison to the control, the deletion of *ORF19.1345Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1345Δ + B. subtilis* vs *ORF19.1345Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1345Δ + B. subtilis (solid, red) was the treated *ORF19.1345Δ* knockout mutant. *ORF19.1345Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1345Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1345Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 16). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table. 23). This suggests that, in comparison to the control,

pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1345Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 16). There was a statistically significant p-value of 0.0380 for the Gehan-Breslow test when comparing the two groups. There was no significant p-value for the LogRank test when comparing the two groups (Table. 23). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1345 is a secreted lipase and a member of a differentially expressed lipase gene family with possible roles in nutrition and/or in creating an acidic microenvironment. LIP5 and LIP8 are expressed at all stages of both mucosal and systemic infection of *ORF19.1345*. *ORF19.1345* molecular functions include lipase and triglyceride lipase activity. Biological processes include interspecies interactions between organisms, lipid catabolic processes, and autophagy of mitochondria. Cellular components include an extracellular region. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1365Δ*

Trial 1

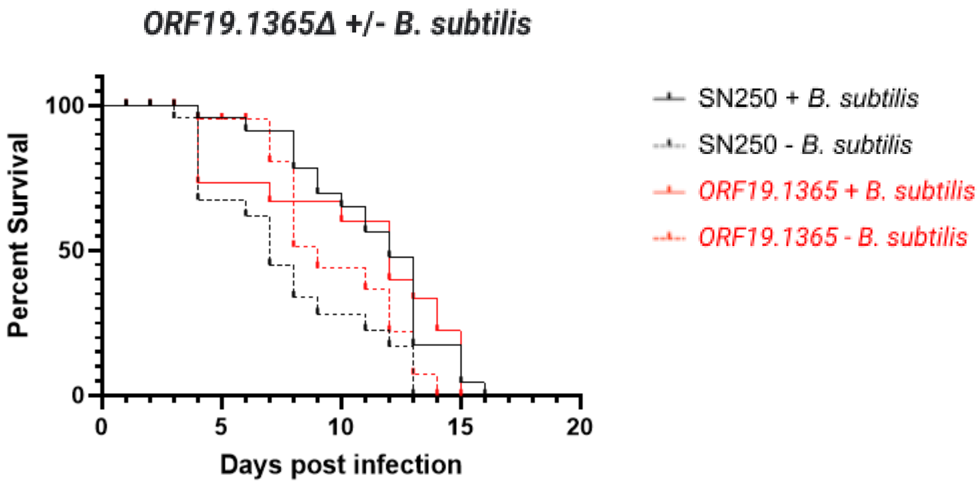

Figure 17: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1365Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1365Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1365Δ* knockout *C. albicans* mutant.

Table 25: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1365Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1365Δ + B. sub.*	*ORF19.1365Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1365Δ + B. sub.*	*ORF19.1365Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0011	NS	-		0.0022	NS	-
SN250 - *B. sub.*	0.0011		-	0.0235	0.0022		-	NS
ORF19.1365Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1365Δ - B. sub.	-	0.0235	NS		-	NS	NS	

Table 26: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1365Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	13
ORF19.1002Δ *C. albicans*	15	14

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1365Δ - B. subtilis*: Deletion of *ORF19.1365* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1365Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1365Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the treated *ORF19.1365Δ* group survived 1 day longer than the untreated group (Fig. 17). There was a statistically significant p-value of 0.0235 for the Gehan-Breslow test when comparing the two groups. There was no statistically significant p-value for the LogRank test when comparing the two groups (Table. 25). This suggests that, in comparison to the control, the deletion of *ORF19.1365Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1365Δ + B. subtilis*: Deletion of *ORF19.1365* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1365Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1365Δ* knockout mutant. The treated SN250 group lived 1 days longer than the treated *ORF19.1365Δ* knockout mutant group (Fig. 17). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 25). This suggests that, in comparison to the control, the deletion of *ORF19.1365Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1365Δ* + *B. subtilis* vs *ORF19.1365Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1365Δ + *B. subtilis* (solid, red) was the treated *ORF19.1365Δ* knockout mutant. *ORF19.1365Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1365* knockout mutant. The comparison between the treated and untreated *ORF19.1365Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 17). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table 25). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1365Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 17). There was a statistically significant p-value of 0.0011 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0022 for the LogRank test when comparing the two groups (Table 25). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Trial 2

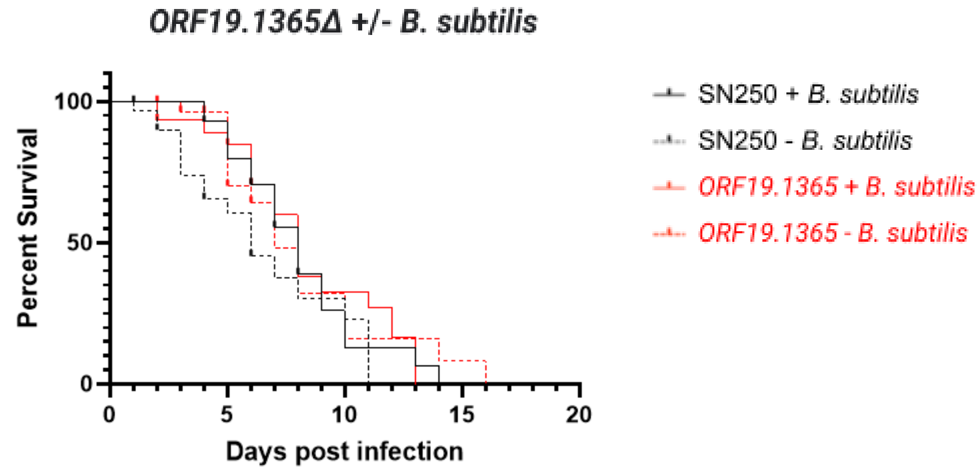

Figure 18: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1365Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1365Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1365Δ* knockout *C. albicans* mutant.

Table 27: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1365Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1365 Δ + B. sub.*	*ORF19.1365 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1365 Δ + B. sub.*	*ORF19.1365 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		NS	NS	-
SN250 - *B. sub.*	0.0245		-	0.0380	NS		-	NS
ORF19.1365Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1365Δ - B. sub.	-	0.0380	NS		-	NS	NS	

Table 28: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1365Δ*

	B. subtilis	OP50
SN250 *C. albicans*	14	11
ORF19.1002Δ C. albicans	13	16

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1365Δ - B. subtilis*: Deletion of *ORF19.1365* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1365Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1365Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the untreated *ORF19.1365Δ* group survived 3 days longer than the treated group (Fig. 18). There was a statistically significant p-value of 0.0380 for the Gehan-Breslow test when comparing the two groups. There was no statistically significant p-value for the LogRank test when comparing the two groups (Table. 27). This suggests that, in comparison to the control, the deletion of *ORF19.1365Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1365Δ + B. subtilis*: Deletion of *ORF19.1365* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1365Δ + B. subtilis* (solid, red) was the treated *ORF19.1365Δ* knockout mutant. The treated SN250 group lived 1 days longer than the treated *ORF19.1365Δ* knockout mutant group (Fig. 18). There was no statistically significant p-value for the Gehan-Breslow or LogRank tests when comparing the two groups (Table. 27). This suggests that, in comparison to the control, the deletion of *ORF19.1365Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1365Δ + B. subtilis* vs *ORF19.1365Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1365Δ + B. subtilis (solid, red) was the treated *ORF19.1365Δ* knockout mutant. *ORF19.1365Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1365Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1365Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group (Fig. 18). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table. 27). This suggests that, in comparison to the control,

pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1365Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 18). There was a statistically significant p-value of 0.0380 for the Gehan-Breslow test when comparing the two groups. There was no significant p-value for the LogRank test when comparing the two groups (Table. 27). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1365 is a putative monooxygenase. This mutation confers hypersensitivity to toxic ergosterol analog and constitutive expression independent of MTL or white-opaque status. *ORF19.1365* molecular functions include monooxygenase activity and oxidoreductase activity. Biological processes include cellular response to drug, carbohydrate transport, and proteolysis. Cellular components are unknown. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1449Δ*

Trial 1

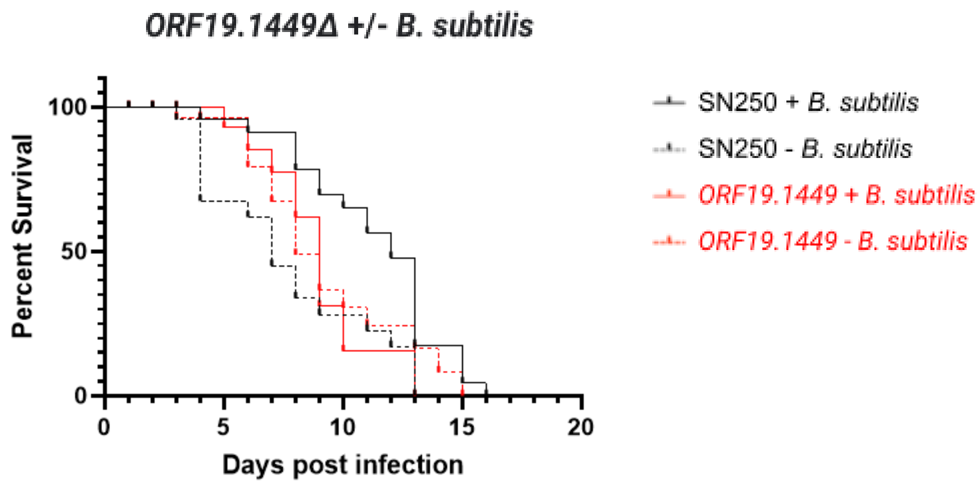

Figure 19: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1449Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1449Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1449Δ* knockout *C. albicans* mutant.

Table 29: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1449Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1449Δ + B. sub.*	*ORF19.1449Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1449Δ + B. sub.*	*ORF19.1449Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0011	0.0175	-		0.0022	0.0094	-
SN250 - *B. sub.*	0.0011		-	NS	0.0022		-	NS
ORF19.1449Δ + B. sub.	0.0175	-		NS	0.0094	-		NS
ORF19.1449Δ - B. sub.	-	NS	NS		-	NS	NS	

Table 30: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1449Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	13
ORF19.1002Δ C. albicans	13	15

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1449Δ - B. subtilis*: Deletion of *ORF19.1449* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1449Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1449Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the untreated *ORF19.1449Δ* group survived 2 days longer than the treated group (Fig. 19). There was no statistically significant p-value for the Gehan-Breslow or the LogRank test when comparing the two groups (Table. 29). This suggests that, in comparison to the control, the deletion of *ORF19.1449Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1449Δ + B. subtilis*: Deletion of *ORF19.1449* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1449Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1449Δ* knockout mutant. The treated SN250 group lived 3 days longer than the treated *ORF19.1449Δ* knockout mutant group (Fig. 19). There was a statistically significant p-value of 0.0175 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0094 for the LogRank test when comparing the two groups (Table. 29). This suggests that, in comparison to the control, the deletion of *ORF19.1449Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1449Δ* + *B. subtilis* vs *ORF19.1449Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1449Δ + *B. subtilis* (solid, red) was the treated *ORF19.1449Δ* knockout mutant. *ORF19.1449Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1449Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1449Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group (Fig. 19). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table. 29). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1449Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 19). There was a statistically significant p-value of 0.0011 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0022 for the LogRank test when comparing the two groups (Table. 29). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Trial 2

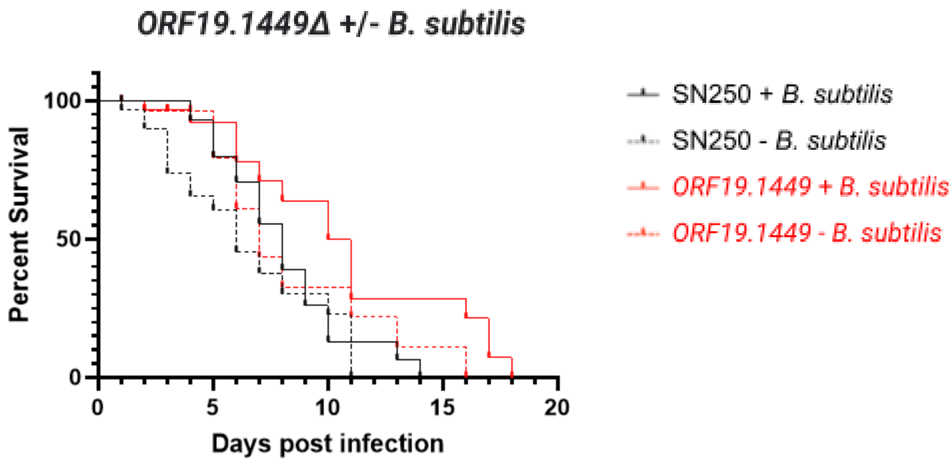

Figure 20: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1449Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1449Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1449Δ* knockout *C. albicans* mutant.

Table 31: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1449Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1449 Δ + B. sub.*	*ORF19.1449 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1449 Δ + B. sub.*	*ORF19.1449 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		NS	0.0350	-
SN250 - *B. sub.*	0.0245		-	NS	NS		-	NS
ORF19.1449Δ + B. sub.	NS	-		NS	0.0350	-		NS
ORF19.1449Δ - B. sub.	-	NS	NS		-	NS	NS	

Table 32: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1449Δ*

	B. subtilis	OP50
SN250 *C. albicans*	14	11
ORF19.1002Δ C. albicans	18	16

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1449Δ - B. subtilis*: Deletion of *ORF19.1449* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1449Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1449Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, and the treated *ORF19.1449Δ* group survived 2 days longer than the untreated group (Fig. 20). There was no statistically significant p-value for the Gehan-Breslow or the LogRank test when comparing the two groups (Table. 31). This suggests that, in comparison to the control, the deletion of *ORF19.1449Δ* does not affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1449Δ + B. subtilis*: Deletion of *ORF19.1449* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1449Δ + B. subtilis* (solid, red) was the treated *ORF19.1449Δ* knockout mutant. The treated SN250 group lived 4 days less than the treated *ORF19.1449Δ* knockout mutant group (Fig. 20). There was no statistically significant p-value for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0350 for the LogRank test when comparing the two groups (Table. 31). This suggests that, in comparison to the control, the deletion of *ORF19.1449Δ* does affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1449Δ + B. subtilis* vs *ORF19.1449Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1449Δ + B. subtilis (solid, red) was the treated *ORF19.1449Δ* knockout mutant. *ORF19.1449Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1449Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1449Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 20). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table. 31). This suggests that, in comparison to the control,

pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1449Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 20). There was a statistically significant p-value of 0.0245 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0350 for the LogRank test when comparing the two groups (Table. 31). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1449 is a protein of unknown function. It is induced in azole-resistant strain that overexpresses MDR1, is present in exponential and stationary growth phase yeast cultures, and is spider biofilm induced. Molecular functions include cyanamide hydratase activity, metal ion binding, and phosphoric diester hydrolase activity. Biological processes include carbohydrate transport and cyanamide metabolic processes. Cellular components are unknown. The homozygous null mutant has abnormal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1502Δ*

Trial 1

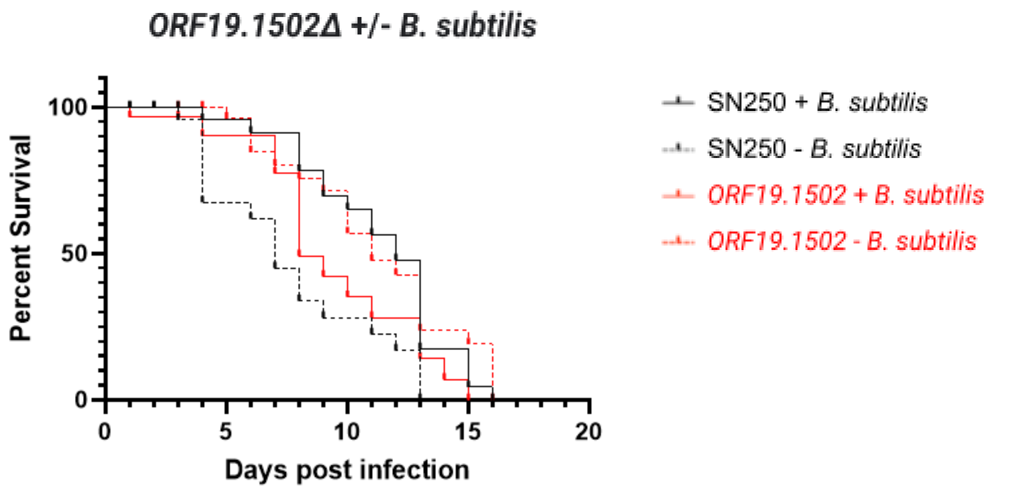

Figure 21: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1502Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1502Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1502Δ* knockout *C. albicans* mutant.

Table 33: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1502Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1502 Δ + B. sub.*	*ORF19.1502 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1502 Δ + B. sub.*	*ORF19.1502 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0011	NS	-		0.0022	NS	-
SN250 - *B. sub.*	0.0011		-	0.0025	0.0022		-	0.0039
ORF19.1502Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1502Δ - B. sub.	-	0.0025	NS		-	0.0039	NS	

Table 34: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1502Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	13
ORF19.1002Δ *C. albicans*	15	16

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1502Δ - B. subtilis*: Deletion of *ORF19.1502* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1502Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1502Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the untreated *ORF19.1502Δ* group survived 1 day longer than the treated group (Fig. 21). There was a statistically significant p-value of 0.0025 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0039 for the LogRank test when comparing the two groups (Table. 33). This suggests that, in comparison to the control, the deletion of *ORF19.1502Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1502Δ* + *B. subtilis*: Deletion of *ORF19.1502* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1502Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1502Δ* knockout mutant. The treated SN250 group lived 3 days longer than the treated *ORF19.1502Δ* knockout mutant group (Fig. 21). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 33). This suggests that, in comparison to the control, the deletion of *ORF19.1502Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1502Δ* + *B. subtilis* vs *ORF19.1502Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1502Δ + *B. subtilis* (solid, red) was the treated *ORF19.1502Δ* knockout mutant. *ORF19.1502Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1502Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1502Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group (Fig. 21). There was no statistically significant p-value for the Gehan-Breslow test or for the LogRank test when comparing the two groups (Table. 33). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1502Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 21). There was a statistically significant p-value of 0.0011 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0022 for the LogRank test when comparing the two groups (Table. 33). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

<u>Trial 2</u>

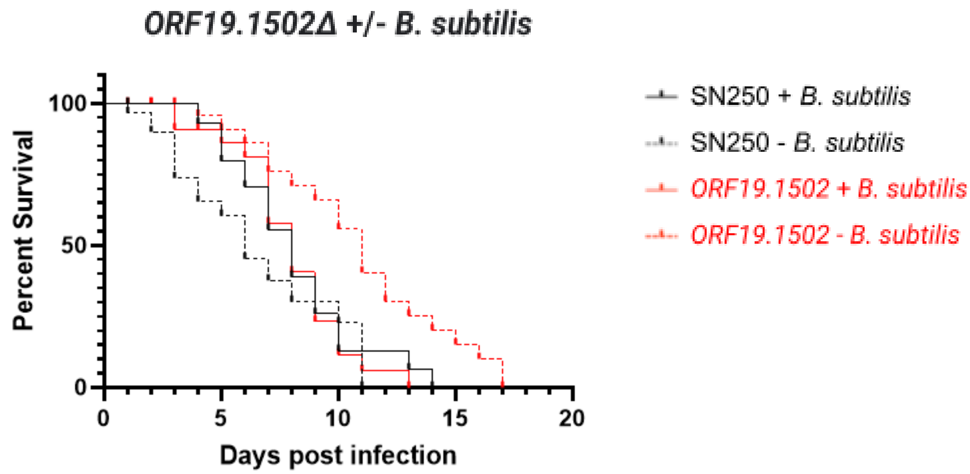

Figure 22: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1502Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1502Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1502Δ* knockout *C. albicans* mutant.

Table 35: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1502Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1502Δ + B. sub.*	*ORF19.1502Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1502Δ + B. sub.*	*ORF19.1502Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		NS	NS	-
SN250 - *B. sub.*	0.0245		-	0.0005	NS		-	0.0005
ORF19.1502Δ + *B. sub.*	NS	-		0.0202	NS	-		0.0042
ORF19.1502Δ - *B. sub.*	-	0.0005	0.0202		-	0.0005	0.0042	

Table 36: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1502Δ*

	B. subtilis	OP50
SN250 *C. albicans*	14	11
ORF19.1002Δ C. albicans	13	17

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1502Δ - B. subtilis*: Deletion of *ORF19.1502* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1502Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1502Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group, but the untreated *ORF19.1502Δ* group survived 4 days longer than the treated group (Fig. 22). There was a statistically significant p-value of 0.0005 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0005 for the LogRank test when comparing the two groups (Table. 35). This suggests that, in comparison to the control, the deletion of *ORF19.1502Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1502Δ + B. subtilis*: Deletion of *ORF19.1502* increases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1502Δ + B. subtilis* (solid, red) was the treated *ORF19.1502Δ* knockout mutant. The treated SN250 group lived 1 day longer than the treated *ORF19.1502Δ* knockout mutant group (Fig. 22). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 35). This suggests that, in comparison to the control, the deletion of *ORF19.1502Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1502Δ + B. subtilis* vs *ORF19.1502Δ - B. subtilis*: Pretreatment with *B. subtilis* increases in vivo virulence

ORF19.1502Δ + B. subtilis (solid, red) was the treated *ORF19.1502Δ* knockout mutant. *ORF19.1502Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1502Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1502Δ* knockout mutants is not similar to the control, as the untreated group lived longer than the treated group (Fig. 22). There was a statistically significant p-value of 0.0202 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0042 for the LogRank test when comparing the two groups (Table. 35). This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1502Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 22). There was a statistically significant p-value of 0.0245 for the Gehan-Breslow test when comparing the two groups. There was no significant p-value for the LogRank test when comparing the two groups (Table. 35). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1502 has domains with predicted aminoacyl-tRNA hydrolase activity. Molecular functions include aminoacyl-tRNA hydrolase activity. Biological processes and cellular components are unknown. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a decreased rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Comparison of treated and untreated wild type *Candida albicans* and mutant *ORF19.1586Δ*

Trial 1

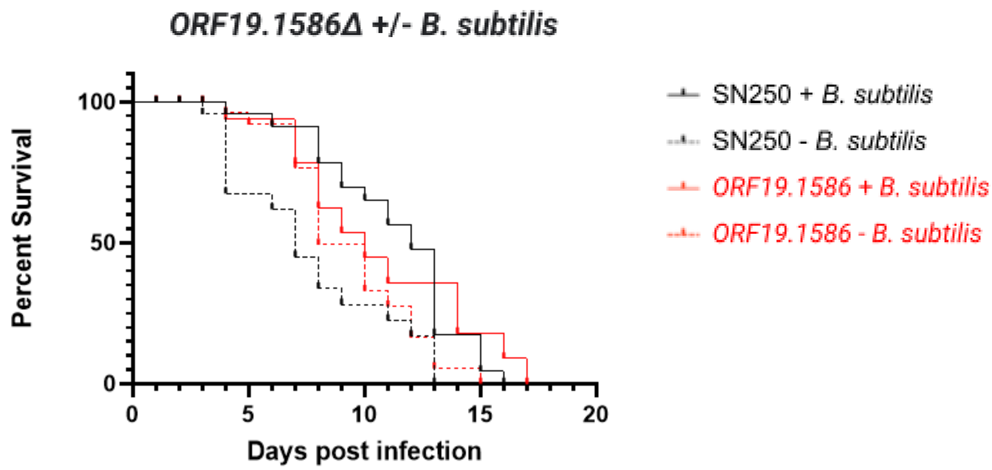

Figure 23: Trial 1 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1586Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1586Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1586Δ* knockout *C. albicans* mutant.

Table 37: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1586Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1586 Δ + B. sub.*	*ORF19.1586 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1586 Δ + B. sub.*	*ORF19.1586 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0011	NS	-		0.0022	NS	-
SN250 - *B. sub.*	0.0011		-	0.0424	0.0022		-	NS
ORF19.1586Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1586Δ - B. sub.	-	0.0424	NS		-	NS	NS	

Table 38: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1586Δ*

	B. subtilis	OP50
SN250 *C. albicans*	16	13
ORF19.1002Δ C. albicans	17	15

(I) SN250 - *B. subtilis* vs *ORF19.1586Δ - B. subtilis*: Deletion of *ORF19.1586* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1586Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1586Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group and the treated *ORF19.1586Δ* group survived 2 days longer than the untreated group (Fig. 23). There was a statistically significant p-value of 0.0424 for the Gehan-Breslow when comparing the two groups. There was no statistically significant p-value for the LogRank test when comparing the two groups (Table. 37). This suggests that, in comparison to the control, the deletion of *ORF19.1586Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(II) SN250 + *B. subtilis* vs *ORF19.1586Δ + B. subtilis*: Deletion of *ORF19.1586* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1586Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1586Δ* knockout mutant. The treated SN250 group lived 1 day less than the treated *ORF19.1586Δ* knockout mutant group (Fig. 23). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 37). This suggests that, in comparison to the control, the deletion of *ORF19.1586Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1586Δ + B. subtilis* vs *ORF19.1586Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1586Δ + B. subtilis (solid, red) was the treated *ORF19.1586Δ* knockout mutant. *ORF19.1586Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1586Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1586Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 23). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 37).This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1586Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 23). There was a statistically significant p-value of 0.0011 for the Gehan-Breslow test when comparing the two groups. There was a significant p-value of 0.0022 for the LogRank test when comparing the two groups (Table. 37). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Trial 2

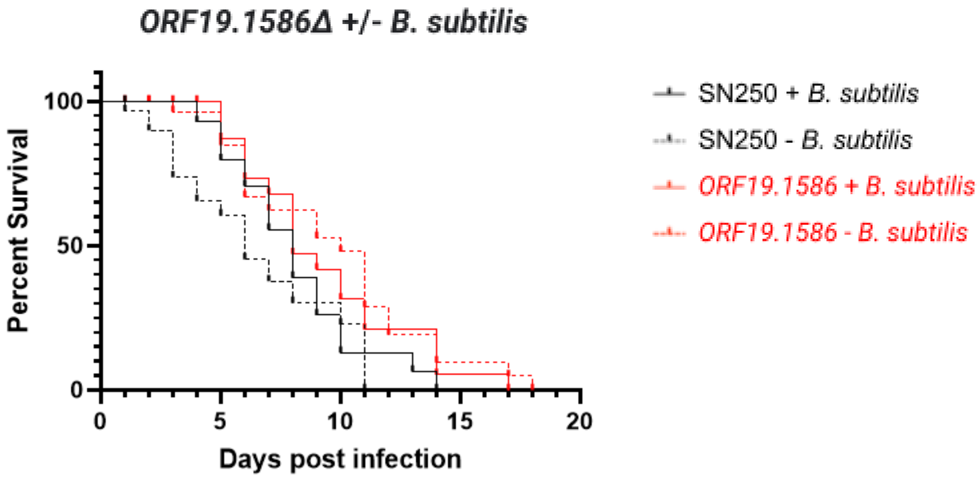

Figure 24: Trial 2 of the survival assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.1586Δ*. The x-axis represents the day post-infection,and the y-axis represents the percent survival rate of the *C. elegans.* The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.1586Δ* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.1586Δ* knockout *C. albicans* mutant.

Table 39: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.1586Δ*. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled "-".

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1586 Δ + B. sub.*	*ORF19.1586 Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.1586 Δ + B. sub.*	*ORF19.1586 Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.0245	NS	-		NS	NS	-
SN250 - *B. sub.*	0.0245		-	0.0066	NS		-	0.0066
ORF19.1586Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.1586Δ - B. sub.	-	0.0066	NS		-	0.0066	NS	

Table 40: The survival time in days of *C. elegans* treated with and without *B. subtilis* for the wildtype *Candida albicans* and mutant *ORF19.1586Δ*

	B. subtilis	OP50
SN250 *C. albicans*	14	11
ORF19.1002Δ C. albicans	17	18

(Ⅰ) SN250 - *B. subtilis* vs *ORF19.1586Δ - B. subtilis*: Deletion of *ORF19.1586* decreases in vivo virulence

SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. *ORF19.1586Δ - B. subtilis* (dotted, red) was the untreated *ORF19.1586Δ* knockout mutant. The SN250 treated group survived 3 days longer than the untreated group and the untreated *ORF19.1586Δ* group survived 1 day longer than the treated group (Fig. 24). There was a statistically significant p-value of 0.0066 for the Gehan-Breslow when comparing the two groups. There was a statistically significant p-value of 0.0066 for the LogRank test when comparing the two groups (Table. 39). This suggests that, in comparison to the control, the deletion of *ORF19.1586Δ* does affect the virulence of *C. albicans* in the untreated *C. elegans* model.

(Ⅱ) SN250 + *B. subtilis* vs *ORF19.1586Δ + B. subtilis*: Deletion of *ORF19.1586* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the treated wild type SN250 control. *ORF19.1586Δ* + *B. subtilis* (solid, red) was the treated *ORF19.1586Δ* knockout mutant. The treated SN250 group lived 3 days less than the treated *ORF19.1586Δ* knockout mutant group (Fig. 24). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 39). This suggests that, in comparison to the control, the deletion of *ORF19.1586Δ* does not affect the virulence of *C. albicans* in the treated *C. elegans* model.

(Ⅲ) *ORF19.1586Δ + B. subtilis* vs *ORF19.1586Δ - B. subtilis*: Pretreatment with *B. subtilis* does not affect in vivo virulence

ORF19.1586Δ + *B. subtilis* (solid, red) was the treated *ORF19.1586Δ* knockout mutant. *ORF19.1586Δ* - *B. subtilis* (dotted, red) was the untreated *ORF19.1586Δ* knockout mutant. The comparison between the treated and untreated *ORF19.1586Δ* knockout mutants is similar to the control, as the treated group lived longer than the untreated group (Fig. 24). There was no statistically significant p-value for the Gehan-Breslow or the LogRank tests when comparing the two groups (Table. 39). This suggests that, in comparison to the control, pre-exposure to *B.*

subtilis does not affect the survival of *C. elegans* when infected with the *C. albicans* *ORF19.1586Δ* knockout mutant.

(Ⅳ) SN250 + *B. subtilis* vs SN250 - *B. subtilis*: Pretreatment with *B. subtilis* decreases in vivo virulence

SN250 + *B. subtilis* (solid, black) was the *B. subtilis* treated wild type SN250 control. SN250 - *B. subtilis* (dotted, black) was the untreated wild type SN250 control. As the control of this experiment, this data was expected. The SN250 + *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* (Fig. 24). There was a statistically significant p-value of 0.0245 for the Gehan-Breslow test when comparing the two groups. There was no significant p-value for the LogRank test when comparing the two groups (Table. 39). This suggests that pre-exposure to *B. subtilis* does affect the survival of *C. elegans* when infected with SN250 at earlier time points for this control.

Genetic Profile:

ORF19.1586 is a putative phosphatidylinositol-specific phospholipase C (PI-PLC), a predicted type 2 membrane protein, and has no *S. cerevisiae* ortholog. It plays a role in, and is regulated by, filamentation and Hap43p and is almost identical to *ORF19.5797*. Molecular functions include phosphatidylinositol phospholipase C activity. Biological processes include cellular response to biotic stimulus and starvation, filamentous growth, carbohydrate transport, and lipid metabolic processes. Cellular components include an integral component of the membrane. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

Discussion

Of the 10 single gene knockout *Candida albicans* mutants we studied, 6 showed a significant difference in virulence compared to the SN250 in both trials. Out of those 6, 4 showed consistent, comparable data among the trials. Out of the 10 mutants, 3 mutants resulted in a significant difference in survival of *Caenorhabditis elegans* with and without pre-exposure to *Bacillus subtilis*, but 1 mutant showed this significance in both trials. For *C. albicans* mutants that did not show significance for any comparison, it is possible that the deleted genes did not affect the virulence of the *C. albicans* and were not necessary for *B. subtilis* to have a beneficial effect on the organism.

ORF19.1239

ORF19.1239 is a secreted protein, and when the protein is exogenously expressed it is a substrate for Kex2 processing in vitro. It is fluconazole-regulated and spider biofilm induced. *ORF19.1239* has an unknown molecular function, biological processes, and cellular components. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

We saw that the *ORF19.1239Δ* mutant was less virulent than the wildtype without *B. subtilis*, but more virulent than the wildtype with *B. subtilis.* The *ORF19.1239Δ - B. subtilis* survived 3 days longer than the SN250 - *B. subtilis* in the first trial and 1 day longer in the second trial. We saw significance in the Gehan-Breslow p-value of 0.0056 and in the LogRank p-value of 0.0052 in the first trial and only in the LogRank p-value of 0.0289 for the second trial. The *ORF19.1239Δ + B. subtilis* lived 2 days less than the SN250 + *B. subtilis* in the first trial and 4 days less in the second trial. There was significance only in the LogRank p-value of 0.0484 in the first trial and only in the LogRank p-value of 0.0137 in the second trial. This suggests that, in comparison to the control, the *ORF19.1239Δ* mutant does affect the virulence of *C. albicans* in the treated *C. elegans* model at later time points (Table XX), but the benefits of *B. subtilis* on the deletion library mutant are not as strong as on the wildtype. This can potentially be explained due to the fact that this protein is a substrate that is exogenously expressed and allows for Kex2 processing. Kex2 is a protease that shares structural similarities to the bacterial protease, subtilisin, which is obtained from *B. subtilis*. Studies have shown that efficient secretion of SAP2 is Kex2 dependent and there is a correlation between the ability of *C. albicans* strains to secrete SAPs and their ability to cause disease (Newport & Agabian, 1997), (Schaller et al, 2003). We believe that the deletion of *ORF19.1239* prevents Kex2 from binding, resulting in the lack of secretion of SAPs, and ultimately preventing *C. albicans* from causing disease. In this case, the *C. elegans* lived the same amount of time with and without *B. subtilis* when infected with the

ORF19.1239Δ mutant because the *B. subtilis* was not actively fighting infection, and therefore was not making a positive or negative impact.

ORF19.1239Δ + *B. subtilis* survived the same amount of time as the *ORF19.1239Δ* - *B. subtilis* in the first and second trials. We saw significance in the Gehan-Breslow p-value of 0.0379 in the first trial but no significance in either test for the second trial. This suggests, in comparison to the control, *B. subtilis* does not improve the survival of *C. elegans* infected with *ORF19.1239Δ* at later points (Table XX). This does not support the understanding, modeled by the SN250 control, that *B. subtilis* improves survival of *C. elegans*. The decreased efficacy of the *B. subtilis* on the mutant compared to the wildtype correlates to the results seen here, as the addition of *B. subtilis* does not have any significant effect on the survival of *C. elegans* infected with *ORF19.1239Δ.*

ORF19.1345

ORF19.1345 is a secreted lipase and a member of a differentially expressed lipase gene family with possible roles in nutrition and/or in creating an acidic microenvironment. LIP5 and LIP8 are expressed at all stages of both mucosal and systemic infection of *ORF19.1345*. *ORF19.1345* molecular functions include lipase and triglyceride lipase activity. Biological processes include interspecies interactions between organisms, lipid catabolic processes, and autophagy of mitochondria. Cellular components include an extracellular region. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

We saw that the *ORF19.1239Δ* mutant was less virulent than the wildtype. *ORF19.1239Δ* - *B. subtilis* survived 3 days longer than SN250 - *B. subtilis* in the first trial and 4 days longer in the second trial. There was significance in the Gehan-Breslow p-value of 0.0172 in the first trial and a Gehan-Breslow p-value of 0.0082 and a LogRank p-value of 0.0311 in the second trial. *ORF19.1345Δ* + *B. subtilis* survived 2 days longer than SN250 + *B. subtilis* in both trials. There was significance in the Gehan-Breslow p-value of 0.0083, but no significance in the second trial. This can potentially be explained as it has been shown that lack of LIP8 results in lesser virulence in *C. albicans*, which supports our results of this deletion mutant showing decreased virulence (Gácser et al, 2007).

ORF19.1345Δ + *B. subtilis* lived 2 days longer than *ORF19.1345Δ* - *B. subtilis* in the first trial and 1 day longer in the second trial. There was no significance found in either trial. This does not support the understanding, modeled by the SN250 control, that *B. subtilis* improves survival of *C. elegans*.

ORF19.1502

ORF19.1502 has domains with predicted aminoacyl-tRNA hydrolase activity. Molecular functions include aminoacyl-tRNA hydrolase activity. Biological processes and cellular components are unknown. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a decreased rate of vegetative growth (Skrzypek, M. S. et al, 2017).

We saw that the *ORF19.1502Δ* mutant was less virulent than the wildtype without *B. subtilis*, but more virulent than the wildtype with *B. subtilis*. *ORF19.1502Δ- B. subtilis* survived 3 days longer than SN250 - *B. subtilis* in the first trial with significance in the Gehan-Breslow p-value of 0.0025 and significance in the LogRank test with a p-value of 0.0039. In the second trial, *ORF19.1502Δ- B. subtilis* survived 6 days longer than SN250 - *B. subtilis* with significance in the Gehan-Breslow with a p-value of 0.0005 and in the LogRank test with a p-value of 0.0005. *ORF19.1502Δ* + *B. subtilis* survived one day less than the SN250 + *B. subtilis* in trial 1 and showed no significance in either test. In trial 2, *ORF19.1502Δ* + *B. subtilis* survived 1 day less than the SN250 + *B. subtilis* with significance in the Gehan-Breslow with a p-value of 0.0202 and in the LogRank test with a p-value of 0.0042. These results show that the addition of *B. subtilis* is less effective on the mutant than on the wildtype. The reason for these results could be due to the loss of aminoacyl-tRNA hydrolase activity. Aminoacyl-tRNAs are substrates for translation and are pivotal in determining how the genetic code is interpreted. Hydrolases are the group of enzymes that catalyze bond cleavages by a reaction with water. Without this ability to break bonds and assist in translation, the likelihood of interpretation of genetic code being incorrect increases. Incorrect interpretation of genetic code could be the reason that *B. subtilis* behaves in an unexpected way with the *ORF19.1502Δ* mutant. (Ibba & Söll, 2000, Alcántara, Hernaiz, & Sinisterra, 2011)

ORF19.1502Δ + *B. subtilis* survived 1 day less than *ORF19.1502Δ* - *B. subtilis* in the first trial, though there was no significance for either the Gehan-Breslow or LogRank tests. In the second trial, *ORF19.1502Δ* + *B. subtilis* survived 4 days less than *ORF19.1502Δ* - *B. subtilis*, and there were significance values for the Gehan-Breslow test of 0.0202 and the LogRank test of 0.0042. This suggests, in comparison to the control, *B. subtilis* does not improve the survival of *C. elegans* infected with *ORF19.1502Δ* at later points (Table XX). This does not support the understanding, modeled by the SN250 control, that *B. subtilis* improves survival of *C. elegans*. These results are similar to that of *ORF19.1239Δ*, as they show a difference in virulence based on the presence of *B. subtilis.* The decreased efficacy of the *B. subtilis* on the mutant compared to the wildtype correlates to the results seen here, as the addition of *B. subtilis* does not have any significant effect on the survival of *C. elegans* infected with *ORF19.1502Δ.*

ORF19.1586

ORF19.1586 is a putative phosphatidylinositol-specific phospholipase C (PI-PLC), a predicted type 2 membrane protein, and has no *S. cerevisiae* ortholog. It plays a role in, and is regulated by, filamentation and Hap43p and is almost identical to *ORF19.5797*. Molecular functions include phosphatidylinositol phospholipase C activity. Biological processes include cellular response to biotic stimulus and starvation, filamentous growth, carbohydrate transport, and lipid metabolic processes. Cellular components include an integral component of the membrane. The homozygous null mutant has normal colony appearance, normal competitive fitness, and a normal rate of vegetative growth (Skrzypek, M. S. et al, 2017).

We saw that the *ORF19.1586Δ* mutant was less virulent than the wildtype. *ORF19.1586Δ* - *B. subtilis* survived 2 days longer than SN250 - *B. subtilis* in the first trial, with significance in only the Gehan-Breslow test with a p-value of 0.0424. In the second trial, *ORF19.1586Δ* - *B. subtilis* survived 7 days longer than SN250 - *B.* subtilis, with significance values of 0.0066 in both the Gehan-Breslow and LogRank tests. *ORF19.1586Δ* + *B. subtilis* survived 1 day longer than the SN250 + *B. subtilis* in the first trial and showed no significance in either test. In the second trial, *ORF19.1586Δ* + *B. subtilis* survived 3 days longer than the SN250 + *B. subtilis*, but again there was no significance for either test. These results are most likely due to the deletion of *ORF19.1586* resulting in a decrease in filamentation of *C. albicans*, resulting in decreased virulence.

ORF19.1586Δ + *B. subtilis* survived 2 days longer than *ORF19.1502Δ* - *B. subtilis* in the first trial, though there was no significance for either the Gehan-Breslow or LogRank tests. In the second trial, *ORF19.1586Δ* + *B. subtilis* survived 1 day less than *ORF19.1502Δ* - *B. subtilis*, and there was no significance for either test. This suggests that, in comparison to the control, pre-exposure to *B. subtilis* does not affect the survival of *C. elegans* when infected with the *C. albicans ORF19.1586Δ* knockout mutant. This does not support the understanding, modeled by the SN250 control, that *B. subtilis* improves survival of *C. elegans*.

General Implications

When comparing the wild type to various gene knockout *C. albicans* mutants, we observed three different outcomes: (1) Mutant knockout had decreased virulence (2) mutant knockout had increased virulence and (3) mutant knockout had no change in virulence. In certain situations, the virulence of the mutant was altered by the presence of *B. subtilis,* or the lack thereof. The knockout mutants that showed a change in virulence are essential to *C. albicans* pathogenicity and are quality candidates for follow up studies. When comparing the *B. subtilis* treated groups to the untreated groups, we observed three different outcomes: (1) *B. subtilis* decreased *C. elegans* survival (2) *B. subtilis* increased *C. elegans* survival (3) *B. subtilis* had no effect on *C. elegans* survival. In some cases, *B. subtilis* was beneficial for the survival of *C. elegans*, but more often, *B. subtilis* was detrimental or had no effect on the survival of *C. elegans*. As a result, follow up studies on the molecular interaction of *B. subtilis* and these knockout mutants should be conducted to further understand the mechanism of *B. subtilis* as a probiotic.

In this study, about 2% of the single gene knockout *C. albicans* mutants of the extensive library from Dr. Suzanne Noble lab were assayed and analyzed. Antifungal drugs are important for fighting infections by *C. albicans*, but this study, and the future ones to come, are important in analyzing the probiotic characteristics of *B. subtilis*, and how probiotics can be used to treat *C. albicans* infection without drug administration.

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Appendices

Appendix Ⅰ: Survival Assay Protocol

DAY 1

1. Streak wild type *B. subtilis* from freezer stock on LB plate, grow overnight in incubator

DAY 2

1. Grow liquid cultures of wild type *B. subtilis* in Tryptic Soy Broth (5mL) (TSB) overnight at 30°C in a roller drum.
2. Seed NGM plate with 120µL OP50/Transfer 10 worms to plate. Leave for two days

DAY 3

1. Streak mutant *C. albicans* on YPD plate from freezer stock, grow overnight in incubator
2. Streak wild type *C. albicans* on YPD plate from well plate, grow overnight in incubator
3. Streak *B. subtilis* on LB plate from liquid culture, grow overnight in incubator

DAY 4

1. Grow liquid cultures of wild type *B. subtilis* in Tryptic Soy Broth (5mL) (TSB) overnight at 30°C in a roller drum.

DAY 5

1. Standardize liquid *B. subtilis* to 8 x 10^8
    1. Centrifuge 500µL of liquid culture (10 mins, 3,200 rpm).
    2. Aspirate supernatant resuspend in 500µL diH_2O, centrifuge for (5 mins, 3,200 rpm).
    3. Aspirate supernatant resuspended in 500µL diH_2O.
    4. Blank cuvette with 1000µL diH_2O
    5. Measure OD600-100µL of pellet and 900µL of diH2O in cuvette.
    6. Dilute original liquid culture to an OD600 of 1.0 (1mL total)
2. Seed NGM plates with OP50 and liquid *B. subtilis* (120µL). Leave on the counter overnight. 2 of each, four in total.

DAY 6

Start Egg Preparation.

1. Wash the plate with M9 buffer (10 mL, 2x 5 mL)
2. Centrifuge (1.5 mins, 3,500 rpm). Aspirate supernatant, leave 1 m

3. Resuspend in 1:4 bleach dilution with 0.25 M NaOH. Invert gently for NO MORE THAN 2 MINS, check under microscope. (1 mL 0.25 M NaOH and 4 mL 10% bleach (5mL total)).
4. Centrifuge 1.5 mins at 2,500 rpm. Aspirate supernatant (leave a little), resuspend in 10mL M9 buffer.
5. Centrifuge for 1.5 mins at 2,500 rpm
6. Aspirate supernatant resuspend in 200µL of M9 buffer.
7. Seed increments of 20µL to the four plates until there are enough eggs on each plate.
8. Let grow and hatch for a day.
9. Make liquid cultures of wild type *C. albicans* in YPD (2.5 diH_2O, 2.5 YPD) overnight at 30°C in a roller-drum.
10. Make liquid cultures mutant strains of *C. albicans* YPD (2.5 diH_2O, 2.5 YPD) overnight at 30°C in a roller-drum.

DAY 7

Leave eggs alone today

1. Standardize OD600 of *C. albicans* liquid cultures to 1.89×10^7
    1. Centrifuge 500µL of liquid culture (10 mins, 3,200 rpm).
    2. Aspirate supernatant resuspend in 500µL diH_2O, centrifuge for (5 mins, 3,200 rpm).
    3. Aspirate supernatant resuspend in 500µL diH_2O.
    4. Blank cuvette with 1000µL diH_2O
    5. Measure OD600-100µL of pellet and 900µL of diH2O in cuvette.
    6. Dilute original liquid culture to an OD600 of 1.0 (1mL total)
2. Seed 2 NGM plates of wild type and 2 NGM plates of mutant (120µL)
3. Leave plates overnight on countertop

DAY 8

1. Transfer as close to 30 worms from egg prep onto each plate from day 5 (1 OP50 and 1 *B. subtilis* for mutant, same for wild type).
   THIS IS DAY 0 IN EXCEL SHEET
2. Make liquid cultures of wild type *C. albicans* in YPD (2.5mL diH20, 2.5mL YPD) overnight at 30°C in a roller-drum.
3. Make liquid cultures mutant strains of *C. albicans* YPD (2.5mL diH20, 2.5mL YPD) overnight at 30°C in a roller-drum.

DAY 9

1. Count worms and document in excel
2. Standardize OD600 of *C. albicans* liquid cultures to 1.89 x 10^7
3. Seed 2 NGM plates of wild type and 2 NGM plates of mutant (120µL)

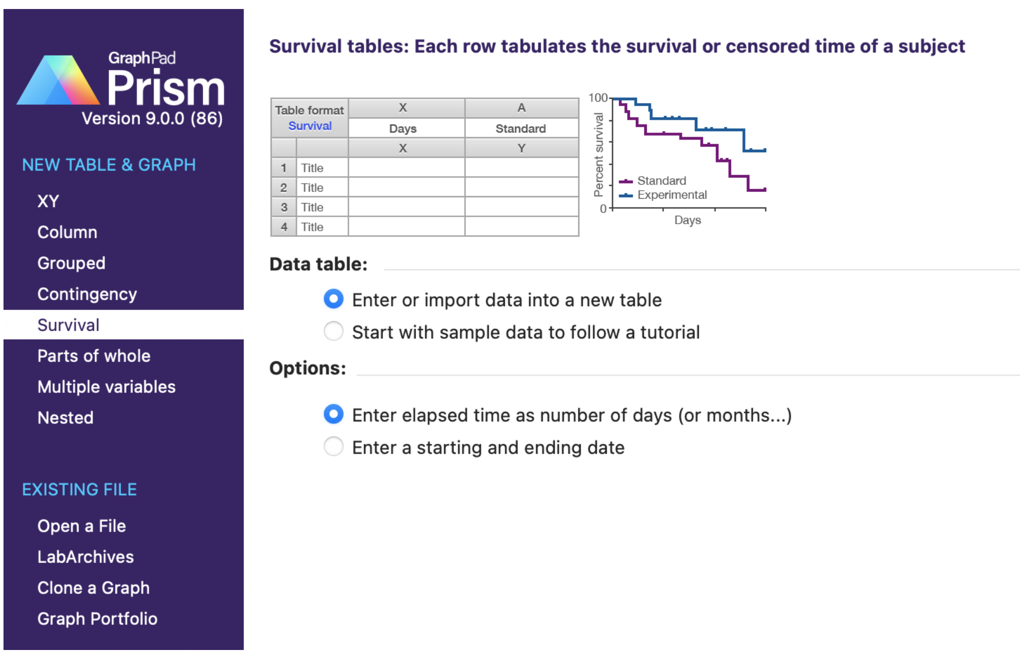
DAY 10

1. Transfer worms to plates seeded from the previous day.
2. Make liquid cultures of *C. albicans* mutant and WT.

Repeat day 9 and day 10 until all worms are dead.

Appendix Ⅱ: GraphPad Data Analysis

1. Choose survival, enter or import data into a new table, and enter elapsed time as a number of days (or months…)



2.
   a. Enter the treatment groups under the group columns.
   b. Enter the days in the X-column
   c. Enter a 1 under each treatment group for each worm that is dead on that day
   d. Enter a 0 under each treatment group for each worm that is missing that day

Table format: Survival		X	Group A	Group B	Group C	Group D
		X Title	*SN250 + B. subtilis*	*SN250 - B. subtilis*	*Mutant + B. subtilis*	*Mutant - B. subtilis*
		X	Y	Y	Y	Y
1	Title	1	1	1	1	1
2	Title	1	1	1	0	1
3	Title	1	1	1	0	1
4	Title	1	1	1	0	1
5	Title	1	1	1	0	0
6	Title	1	1	1		0
7	Title	1	1	0		0
8	Title	1	1	0		0
9	Title	1	1	0		0
10	Title	1	0	0		0
11	Title	1	0	0		
12	Title	1	0			
13	Title	1	0			
14	Title	1	0			
15	Title	2	1		1	1
16	Title	2	1		1	1
17	Title	2	0		0	0
18	Title	2	0		0	0
19	Title	2	0		0	
20	Title	2	0		0	
21	Title	2			0	
22	Title	2			0	
23	Title	2			0	

3. Create a comparison between each treatment group for a total of 6 comparisons.

Results

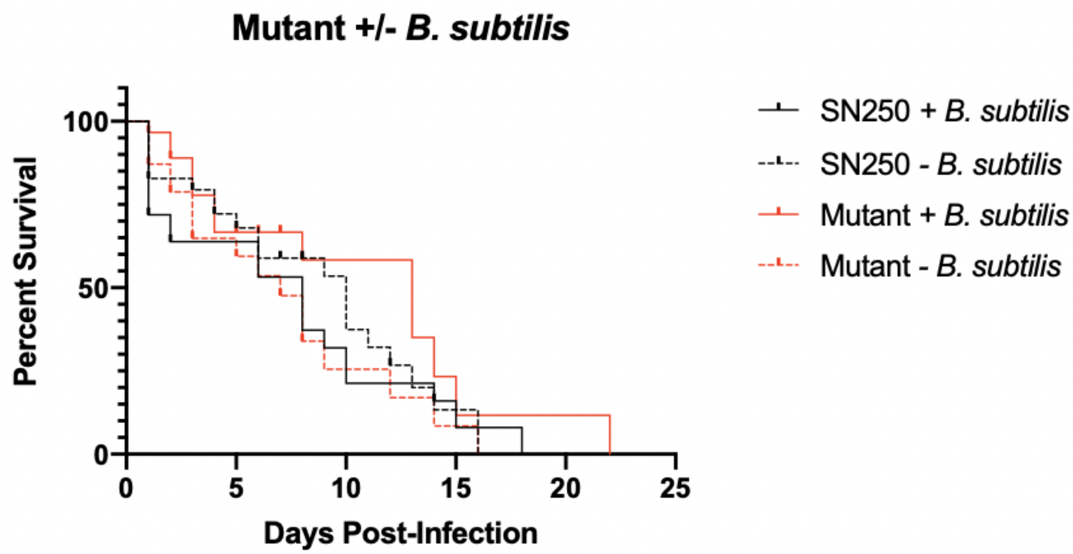
- **SN250 & UPC2 +/- B. subtilis**
- **SN250 +/- B. subtilis**
- **UPC2 +/- B. subtilis**
- **SN250 + B. subtilis vs. UPC2 + B. subtilis**
- **SN250 + B. subtilis vs. UPC2 - B. subtilis**
- **SN250 - B. subtilis vs. UPC2 + B. subtilis**
- **SN250 - B. subilis vs. UPC2 - B. subtilis**

4. Under results in the curve comparison tab, record the significance of the LogRank Test, and significance and p-value of the Gehan-Breslow-Wilcoxon Test.

at risk | **Curve comparison** | Data summary

	Survival Curve comparison	
1	**Comparison of Survival Curves**	
2		
3	**Log-rank (Mantel-Cox) test**	
4	Chi square	3.268
5	df	1
6	P value	0.0706
7	P value summary	ns
8	Are the survival curves sig different?	No
9		
10	**Gehan-Breslow-Wilcoxon test**	
11	Chi square	5.441
12	df	1
13	P value	0.0197
14	P value summary	*
15	Are the survival curves sig different?	Yes

5. For the graph: wild type data black, mutant red, untreated dashed, and treated solid.



Appendix Ⅲ: Completed Assays from 2019-2020 MQP for Aaron Mitchell Green Plate 1

Gene: CJN242

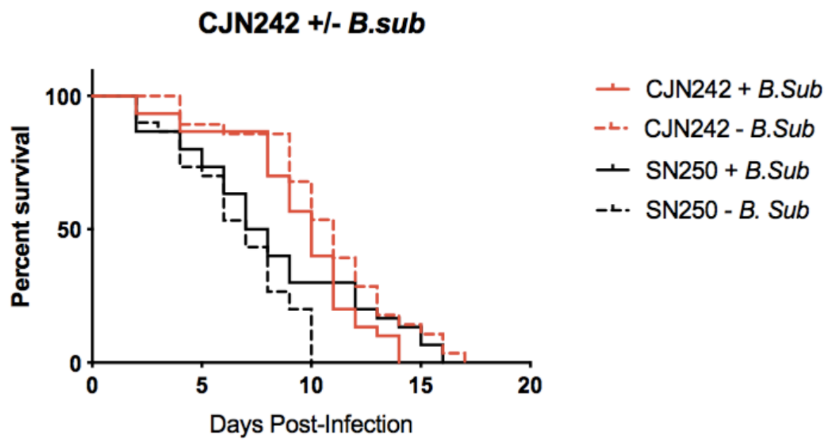


Figure 16: Survival curve of treated and untreated CJN242 *C. albicans* mutant with *B. subtilis*

Table 15: Log-Rank p-value for survival assay of treated and untreated CJN242 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN242 + *B. sub*	CJN242 - *B. sub*
SN250 + *B.sub*		0.0488	No	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN242 + *B. sub*	No	<0.0001		No
CJN242 - *B. sub*	No	<0.0001	No	

Gene: CJN267

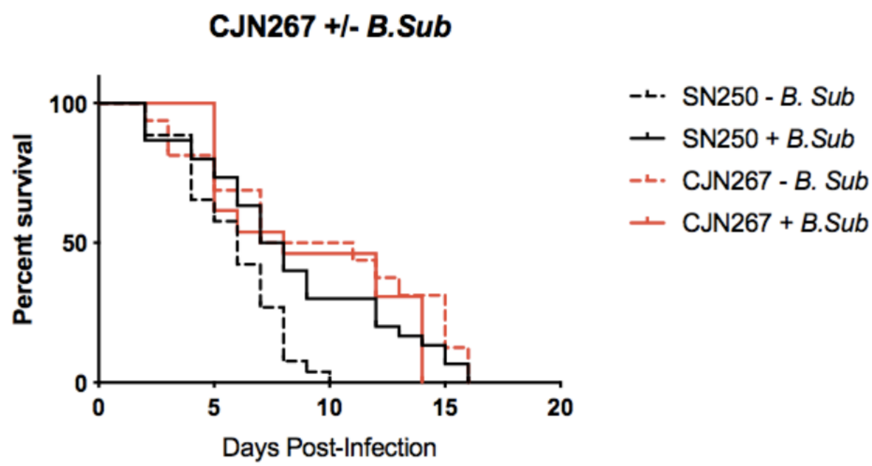


Figure 17: Survival curve of treated and untreated CJN267 *C. albicans* mutant with *B. subtilis*

Table 16: Log-Rank p-value for survival assay of treated and untreated CJN267 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN267 + *B. sub*	CJN267 - *B. sub*
SN250 + *B. sub*		0.0048	No	No
SN250 - *B. sub*	0.0048		0.0041	0.0026
CJN267 + *B. sub*	No	0.0041		No
CJN267 - *B. sub*	No	0.0026	No	

Gene: CJN299

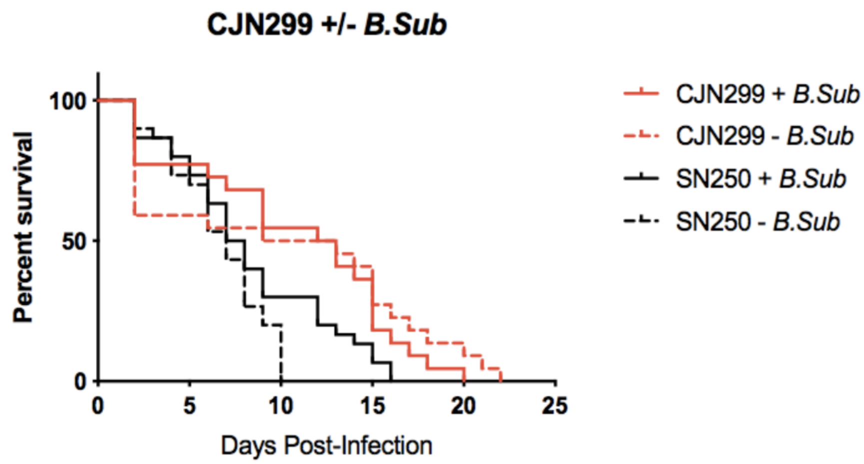


Figure 18: Survival curve of treated and untreated CJN299 *C. albicans* mutant with *B. subtilis*

Table 17: Log-Rank p-value for survival assay of treated and untreated CJN299 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN299 + *B. sub*	CJN299 - *B.sub*
SN250 + *B. sub*		0.0488	0.0287	0.0399
SN250 - *B. sub*	0.0488		0.0003	0.0063
CJN299 + *B. sub*	0.0287	0.0003		No
CJN299 - *B. sub*	0.0399	0.0063	No	

Gene: CJN348

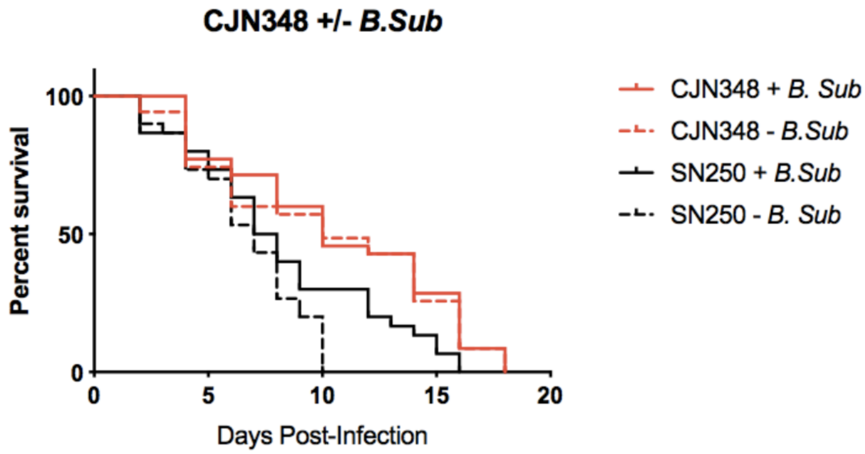


Figure 19: Survival curve of treated and untreated CJN348 *C. albicans* mutant with *B. subtilis*

Table 18: Log-Rank p-value for survival assay of treated and untreated CJN348 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN348 + *B. sub*	CJN348 - *B. sub*
SN250 + *B. sub*		0.0048	0.0109	0.0329
SN250 - *B. sub*	0.0048		<0.0001	0.0002
CJN348 + *B. sub*	0.0109	<0.0001		No
CJN348 - *B. sub*	0.0329	0.0002	No	

Gene: CJN393

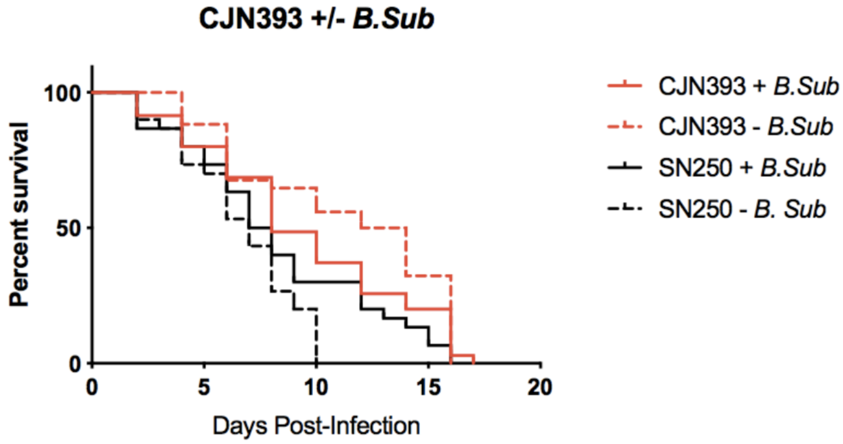


Figure 20: Survival curve of treated and untreated CJN393 *C. albicans* mutant with *B. subtilis*

Table 19: Log-Rank p-value for survival assay of treated and untreated CJN393 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN393 + *B. sub*	CJN393 - *B. sub*
SN250 + *B. sub*		0.0048	No	0.0043
SN250 - *B. sub*	0.0048		0.0010	<0.0001
CJN393 + *B. sub*	No	0.0010		No
CJN393 - *B.sub*	0.0043	<0.0001	No	

Gene: CJN395

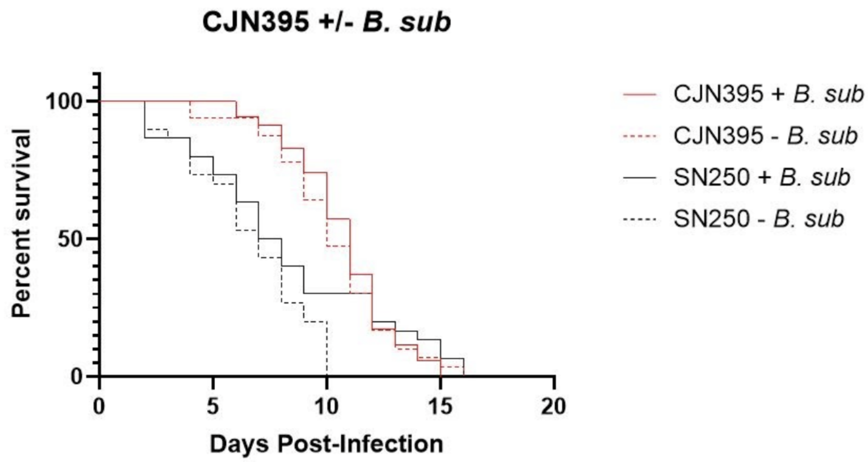


Figure 21: Survival curve of treated and untreated CJN395 *C. albicans* mutant with *B. subtilis*

Table 20: Log-Rank p-value for survival assay of treated and untreated CJN395 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN395 + *B. sub*	CJN395 - *B. sub*
SN250 + *B. sub*		0.0488	No	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN395 + *B. sub*	No	<0.0001		No
CJN395 - *B.sub*	No	<0.0001	No	

Gene: CJN396

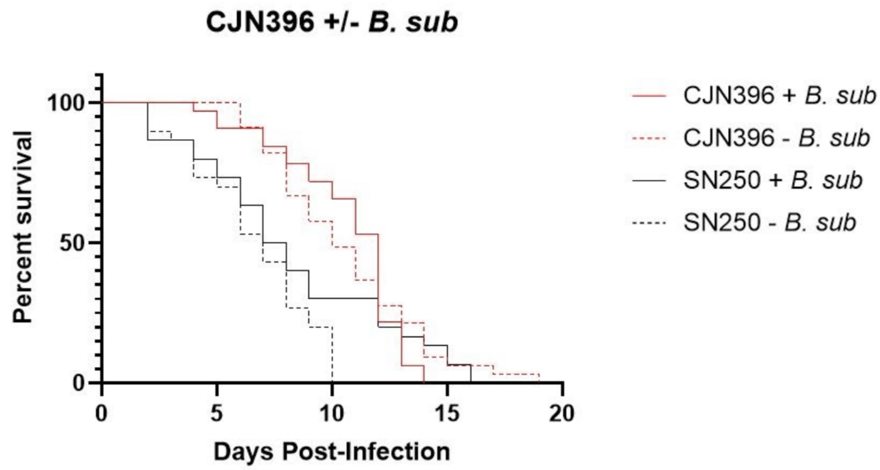


Figure 22: Survival curve of treated and untreated CJN396 *C. albicans* mutant with *B. subtilis*

Table 21: Log-Rank p-value for survival assay of treated and untreated CJN396 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN396 + *B.sub*	CJN396 - *B.sub*
SN250 + *B. sub*		0.0488	No	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN396 + *B. sub*	No	<0.0001		No
CJN396 - *B.sub*	No	<0.0001	No	

Gene: CJN401

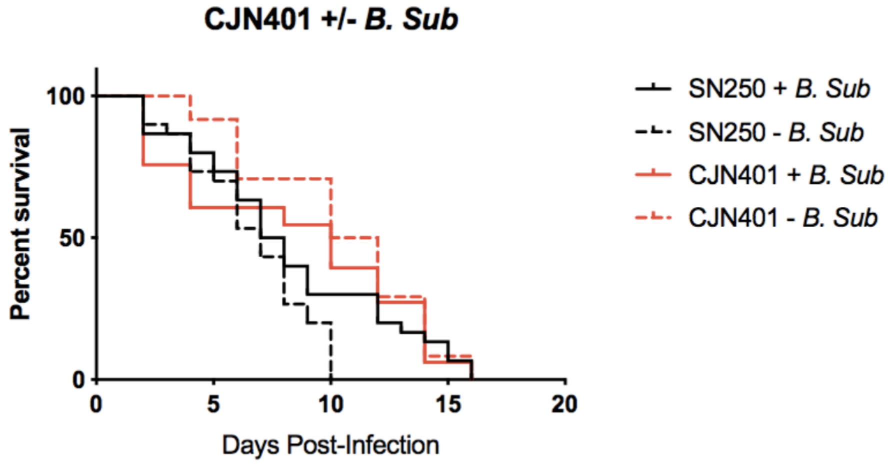


Figure 23: Survival curve of treated and untreated CJN401 *C. albicans* mutant with *B. subtilis*

Table 22: Log-Rank p-value for survival assay of treated and untreated CJN401 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN401 + *B. sub*	CJN401 - *B. sub*
SN250 + *B. sub*		0.0488	No	No
SN250 - *B.sub*	0.0488		0.0030	<0.001
CJN401 + *B. sub*	No	0.0030		No
CJN401 - *B. sub*	No	<0.001	No	

Gene: CJN403

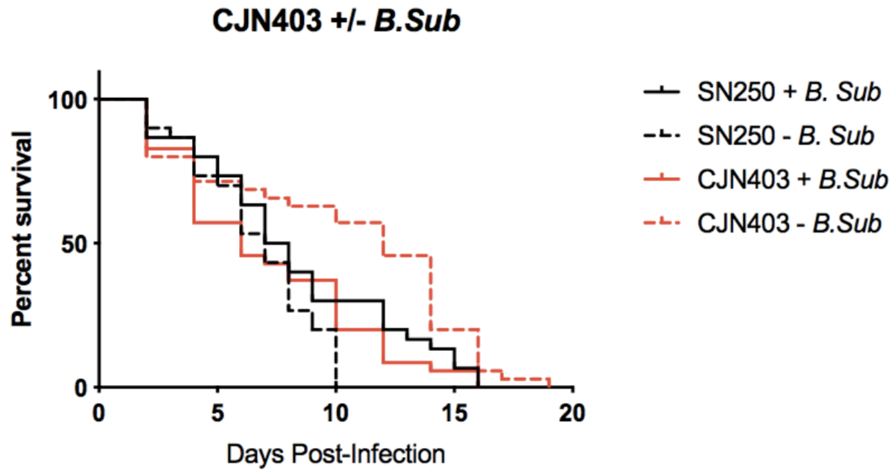


Figure 24: Survival curve of treated and untreated CJN403 *C. albicans* mutant with *B. subtilis*

Table 23: Log-Rank p-value for survival assay of treated and untreated CJN403 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN403 + *B .sub*	CJN403 - *B. sub*
SN250 + *B. sub*		0.0048	No	0.0317
SN250 - *B. sub*	0.0048		No	<0.0001
CJN403 + *B. sub*	No	No		0.0025
CJN403 - *B. sub*	0.0317	<0.0001	0.0025	

Gene: CJN411

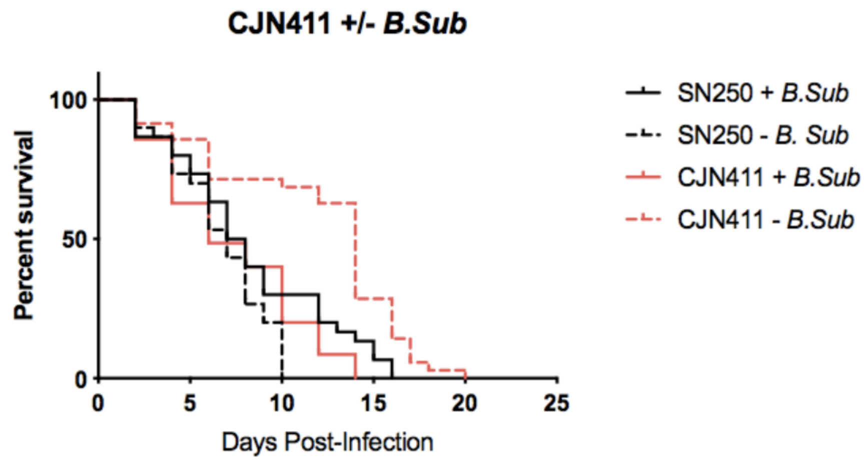


Figure 25: Survival curve of treated and untreated CJN411 *C. albicans* mutant with *B. subtilis*

Table 24: Log-Rank p-value for survival assay of treated and untreated CJN411 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN411 + *B. sub*	CJN411 - *B. sub*
SN250 + *B. sub*		0.0488	No	0.0006
SN250 - *B. sub*	0.0488		No	<0.0001
CJN411 + *B. sub*	No	No		<0.0001
CJN411 - *B. sub*	0.0006	<0.0001	<0.0001	

Gene: CJN419

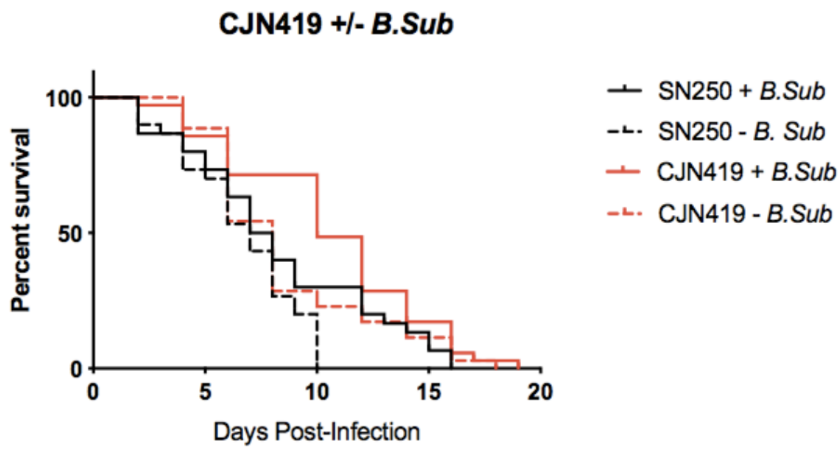


Figure 26: Survival curve of treated and untreated CJN419 *C. albicans* mutant with *B. subtilis*

Table 25: Log-Rank p-value for survival assay of treated and untreated CJN419 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN419 + *B. sub*	CJN419 - *B. sub*
SN250 + *B. sub*		0.0048	0.0357	No
SN250 - *B. sub*	0.0048		<0.0001	0.0374
CJN419 + *B. sub*	0.0357	<0.0001		No
CJN419 - *B. sub*	No	0.0374	No	

Gene: CJN427

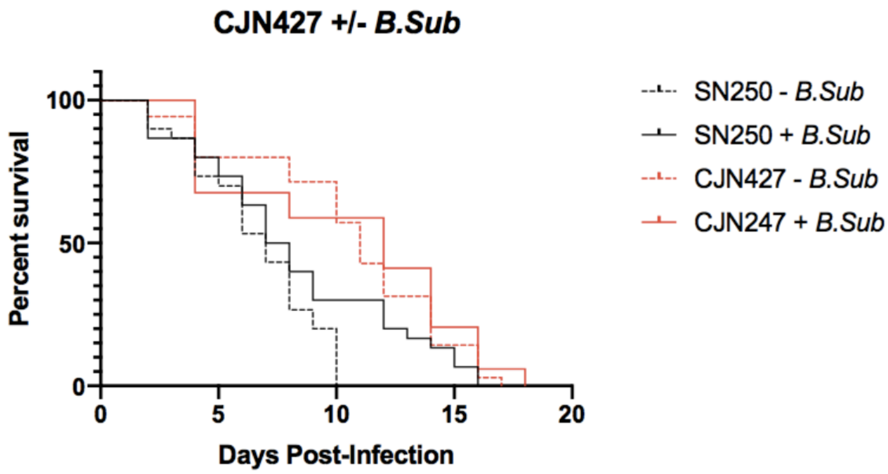


Figure 27: Survival curve of treated and untreated CJN427 *C. albicans* mutant with *B. subtilis*

Table 26: Log-Rank p-value for survival assay of treated and untreated CJN427 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN427 + *B. sub*	CJN427 - *B. sub*
SN250 + *B. sub*		0.0488	0.0278	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN427 + *B. sub*	0.0278	<0.0001		No
CJN427 - *B. sub*	No	<0.0001	No	

Gene: CJN432

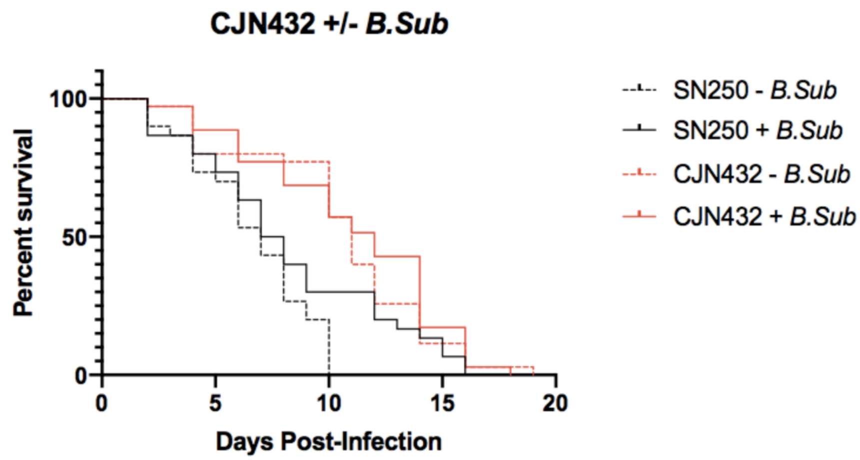


Figure 28: Survival curve of treated and untreated CJN432 *C. albicans* mutant with *B. subtilis*

Table 27: Log-Rank p-value for survival assay of treated and untreated CJN432 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN432 + *B. sub*	CJN432 - *B. sub*
SN250 + *B. sub*		0.0488	0.0174	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN432 + *B. sub*	0.0174	<0.0001		No
CJN432 - *B.sub*	No	<0.0001	No	

Gene: CJN434

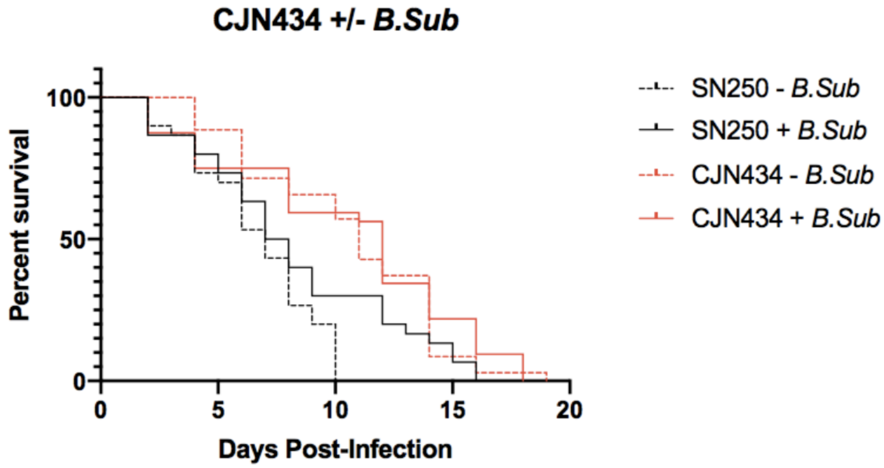


Figure 29: Survival curve of treated and untreated CJN434 *C. albicans* mutant with *B. subtilis*

Table 28: Log-Rank p-value for survival assay of treated and untreated CJN434 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN434 + *B. sub*	CJN434 - *B.sub*
SN250 + *B. sub*		0.0488	0.0303	No
SN250 - *B. sub*	0.0488		<0.0001	<0.0001
CJN434 + *B. sub*	0.0303	<0.0001		No
CJN434 - *B. sub*	No	<0.0001	No	

Gene: CJN442

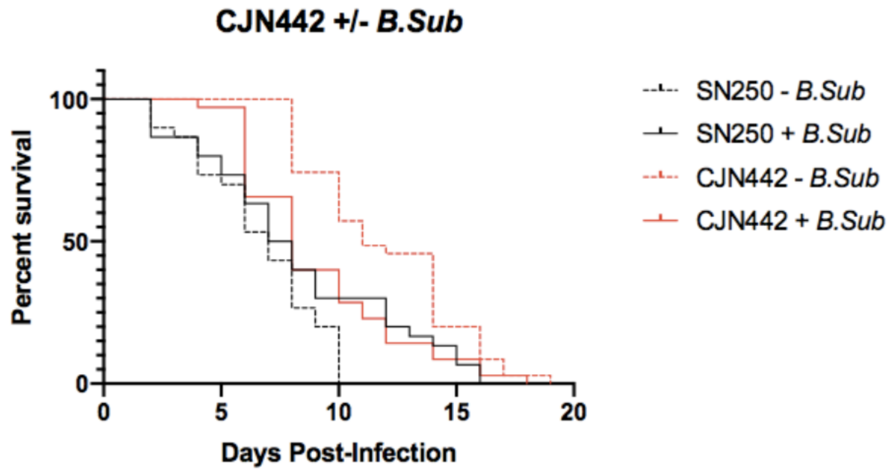


Figure 30: Survival curve of treated and untreated CJN442 *C. albicans* mutant with *B. subtilis*

Table 29: Log-Rank p-value for survival assay of treated and untreated CJN442 *C. albicans* with *B. subtilis*

	SN250 + *B. sub*	SN250 - *B. sub*	CJN442 + *B. sub*	CJN442 - *B. sub*
SN250 + *B. sub*		0.0488	No	0.0008
SN250 - *B. sub*	0.0488		0.0018	<0.0001
CJN442 + *B. sub*	No	0.0018		0.0025
CJN442 - *B. sub*	0.0008	<0.0001	0.0025	

Appendix Ⅳ: Completed Assays from 2020-2021 MQP for Suzanne Noble gene knockout library

Gene: *ORF19.931Δ*

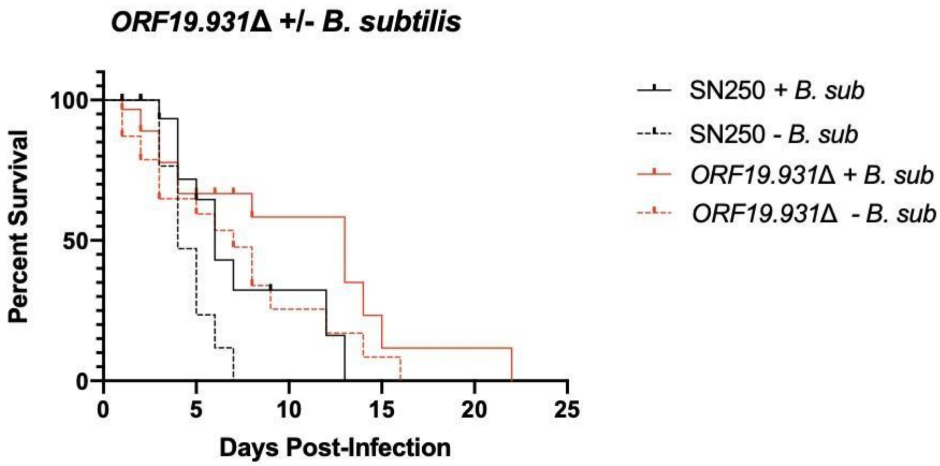


Figure 2: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.931*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.931* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.931* knockout *C. albicans* mutant.

Table 1: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.931*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed are labeled “-”.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.931Δ + B. sub.*	*ORF19.931Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.931Δ+ B. sub.*	*ORF19.9 31Δ - B. sub.*
	Gehan-Breslow p-value				LogRank p-value			
SN250 + *B. sub.*		0.0255	NS	-		0.0093	NS	-
SN250 - *B. sub.*	0.0255		-	NS	0.0093		-	NS
ORF19.931Δ + *B. sub.*	NS	-		NS	NS	-		NS
ORF19.931Δ - *B. sub.*	-	NS	NS		-	-	NS	

Gene: *ORF19.762Δ*

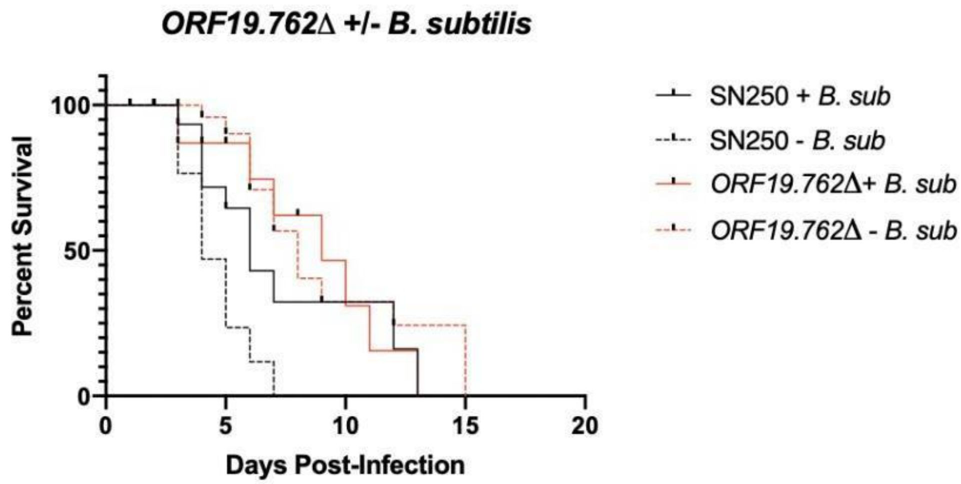


Figure 3: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.762*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.762* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.762* knockout *C. albicans* mutant.

Table 2: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.762*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.762*Δ + *B. sub.*	*ORF19.762*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.762*Δ + *B. sub.*	*ORF19.762*Δ - *B. sub.*
	Gehan-Breslow p-value				LogRank p-value			
SN250 + *B. sub.*		0.0255	NS	-		0.0093	NS	-
SN250 - *B. sub.*	0.0255		-	<0.0001	0.0093		-	<0.0001
*ORF19.762*Δ + *B. sub.*	NS	-		NS	NS	-		NS
*ORF19.762*Δ - *B. sub.*	-	<0.0001	NS		-	<0.0001	NS	

Gene: *ORF19.716Δ*

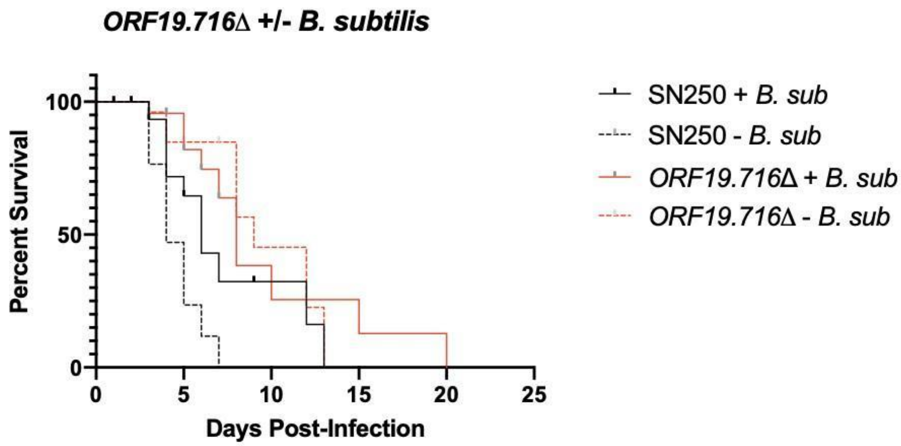


Figure 4: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.716*Δ. The x-axis represents the day post-infection and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.716* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.716* knockout *C. albicans* mutant.

Table 3: Statistical significance of Gehan-Breslow-Wilcoxon and LogRank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.716*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.716Δ + B. sub.*	*ORF19.716Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.716Δ + B. sub.*	*ORF19.716Δ - B. sub.*
	Gehan-Breslow p-value				LogRank p-value			
SN250 + *B. sub.*		0.0255	NS	-		0.0093	NS	-
SN250 - *B. sub.*	0.0255		-	<0.0001	0.0093		-	<0.0001
ORF19.716Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.716Δ - B. sub.	-	<0.0001	NS		-	<0.0001	NS	

Gene: ***ORF19.899Δ***

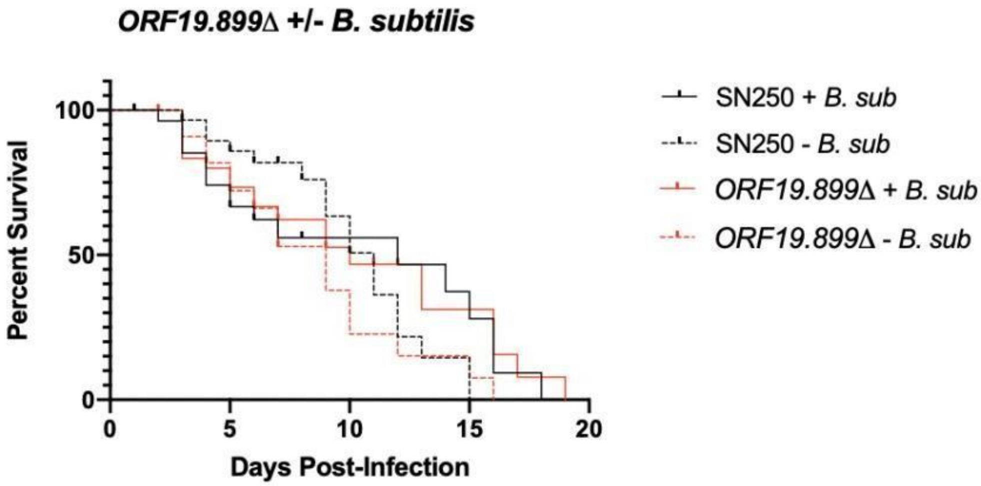


Figure 5: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.899*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.899* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted redline is untreated *ORF19.899* knockout *C. albicans* mutant.

Table 4: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.899*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.899*Δ + *B. sub.*	*ORF19.899*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.899*Δ + *B. sub.*	*ORF19.8 99*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		NS	NS	-		NS	NS	-
SN250 - *B. sub.*	NS		-	NS	NS		-	NS
*ORF19.899*Δ + *B. sub.*	NS	-		NS	NS	-		NS
*ORF19.899*Δ - *B. sub.*	-	NS	NS		-	NS	NS	

Gene: *ORF19.936Δ*

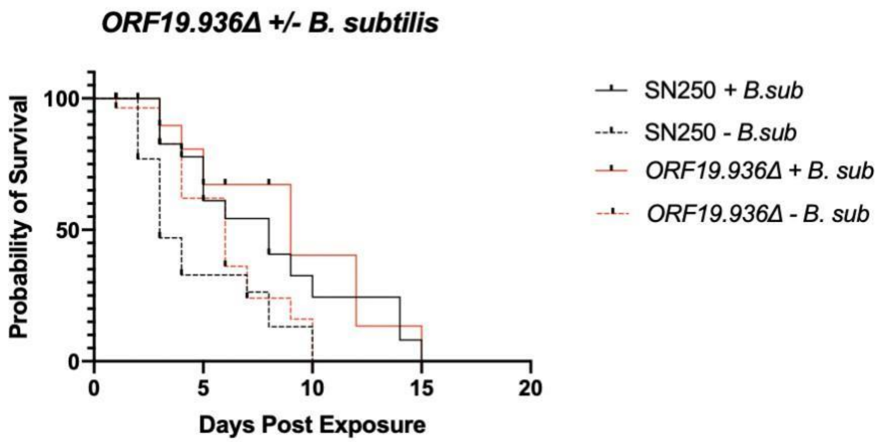


Figure 6: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.936*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.936* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.936* knockout *C. albicans* mutant.

Table 5: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.936*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.936Δ + B. sub.*	*ORF19.936Δ - B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.936Δ + B. sub.*	*ORF19.936Δ - B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		0.0011	NS	-		0.0026	NS	-
SN250 - *B. sub.*	0.0011		-	NS	0.0026		-	NS
ORF19.936Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.936Δ - B. sub.	-	NS	NS		-	NS	NS	

Gene: *ORF19.993Δ*

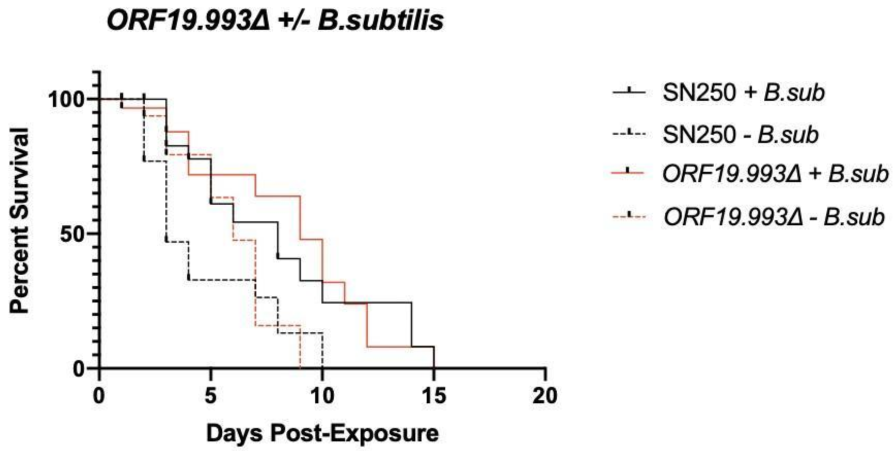


Figure 7: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.993*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.993* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.993* knockout *C. albicans* mutant.

Table 6: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.993*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.993*Δ + *B. sub.*	*ORF19.993*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.993*Δ + *B. sub.*	*ORF19.993*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		0.0011	NS	-		0.0026	NS	-
SN250 - *B. sub.*	0.0011		-	NS	0.0026		-	NS
*ORF19.993*Δ + *B. sub.*	NS	-		NS	NS	-		NS
*ORF19.993*Δ - *B. sub.*	-	NS	NS		-	NS	NS	

Gene: *cip1*

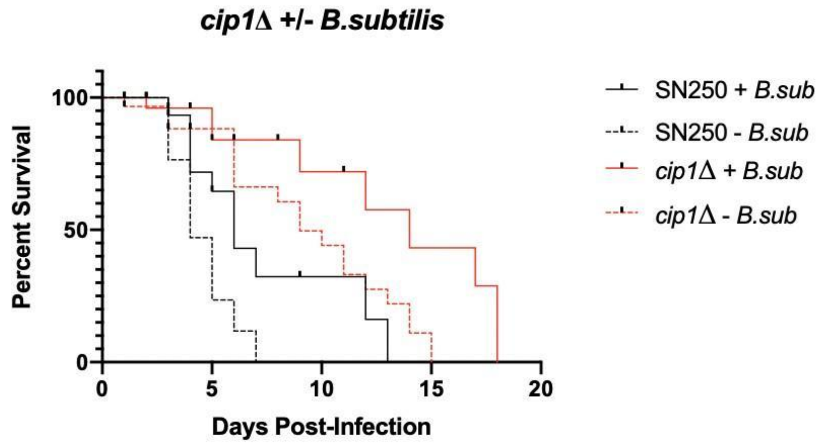


Figure 8: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *cip1*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *cip1* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *cip1* knockout *C. albicans* mutant.

Table 7: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *cip1*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*cip1*Δ + *B. sub.*	*cip1*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*cip1*Δ + *B. sub.*	*cip1*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				Log Rank p-value			
SN250 + *B. sub.*		0.025	NS	-		0.0093	<0.0001	-
SN250 - *B. sub.*	0.025		-	0.0009	0.0093		-	<0.0001
*cip1*Δ + *B. sub.*	NS	-		NS	0.0089	-		0.0195
*cip1*Δ - *B. sub.*	-	NS	NS		-	NS	0.0195	

Gene: ***fad2***

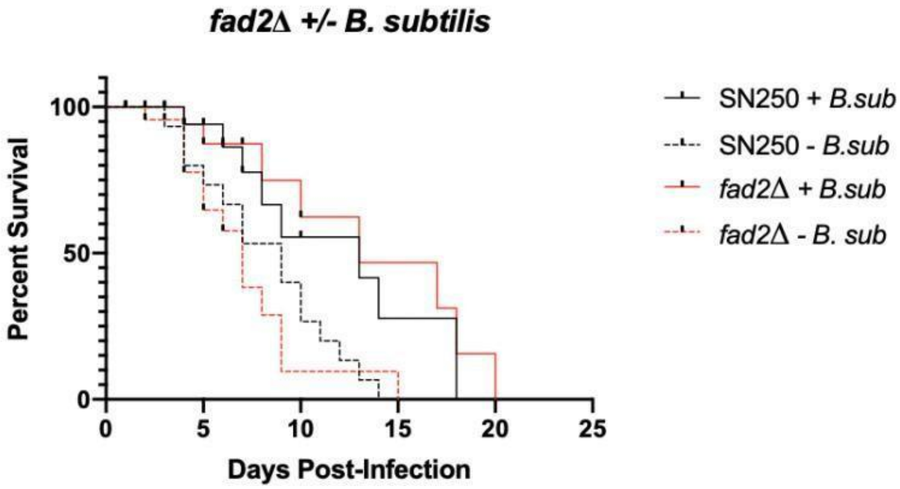


Figure 9: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *fad2*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *fad2* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *fad2* knockout *C. albicans* mutant.

Table 8: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *fad2*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*fad2*Δ + *B. sub.*	*fad2*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*fad2*Δ + *B. sub.*	*fad2*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		NS	NS	-		0.0255	NS	-
SN250 - *B. sub.*	NS		-	NS	0.0255		-	NS
*fad2*Δ+ *B. sub.*	NS	-		0.01	NS	-		0.0019
*fad2*Δ - *B. sub.*	-	NS	0.01		-	NS	0.0019	

Gene: *sld1*

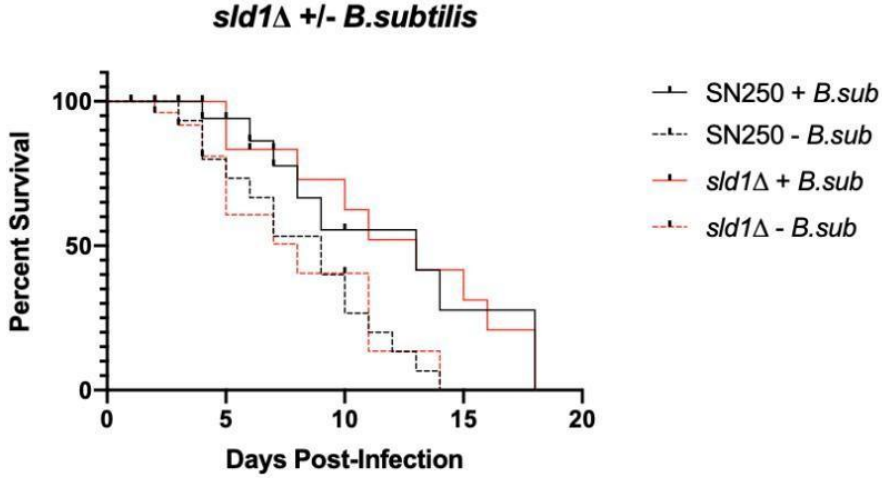


Figure 10: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *sld1*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *sld1* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *sld1* knockout *C. albicans* mutant.

Table 9: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *fad2*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*sld1*Δ + *B. sub.*	*sld1*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*sld1*Δ + *B. sub.*	*sld1*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		NS	NS	-		0.0255	NS	-
SN250 - *B. sub.*	NS		-	NS	0.0255		-	NS
*sld1*Δ + *B. sub.*	NS	-		0.0245	NS	-		0.0182
*sld1*Δ - *B. sub.*	-	NS	0.0245		-	NS	0.0182	

Gene: ***ORF19.278Δ***

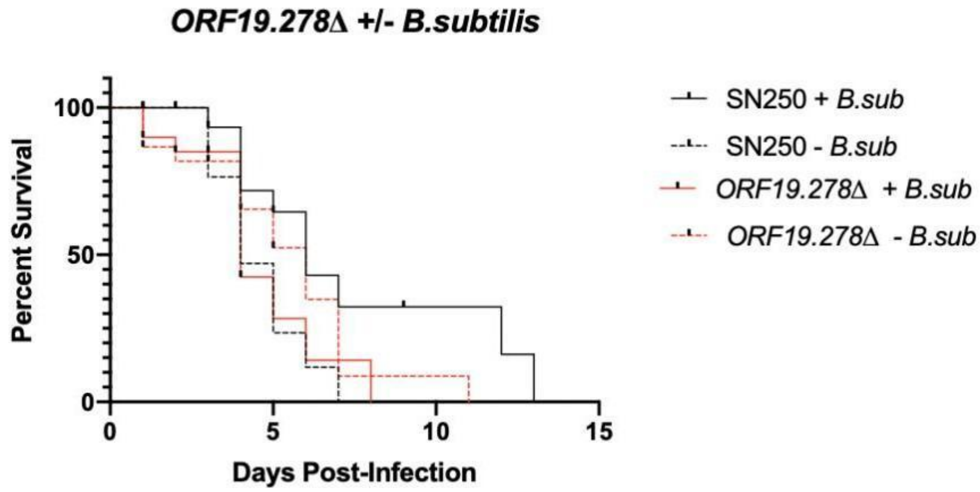


Figure 11: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.278*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *ORF19.278* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.278* knockout *C. albicans* mutant.

Table 10: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.278*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.2 78*Δ + *B. sub.*	*ORF19.2 78*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.2 78*Δ + *B. sub.*	*ORF19.2 78*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		0.02039	0.019	-		0.0093	0.0183	-
SN250 - *B. sub.*	0.02039		-	NS	0.0093		-	NS
*ORF19.278*Δ + *B. sub.*	0.019	-		NS	0.0183	-		NS
*ORF19.278*Δ - *B. sub.*	-	NS	NS		-	NS	NS	

Gene: *nuo2*

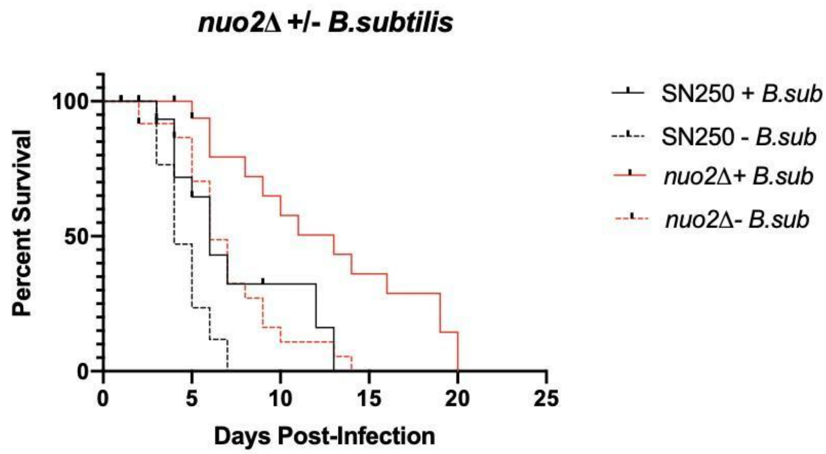


Figure 12: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *nuo2*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *nuo2* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *nuo2* knockout *C. albicans* mutant.

Table 11: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *nuo2*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	nuo2Δ + B. sub.	nuo2Δ - B. sub.	SN250 + *B. sub.*	SN250 - *B. sub.*	nuo2Δ + B. sub.	nuo2 - *B. sub*
	Gehan-Breslow-Wilcoxon p-value				LogRank p-value			
SN250 + *B. sub.*		0.0255	0.0093	-		0.010	0.013	
SN250 - *B. sub.*	0.0255		-	0.0093	0.010		-	-
nuo2Δ + B. sub.	0.0093	-		0.0023	0.01	-		0.006
nuo2Δ - B. sub.	-	0.0093	0.0023		-	0.006	0.0007	0.0007

Gene: ***ORF19.376Δ***

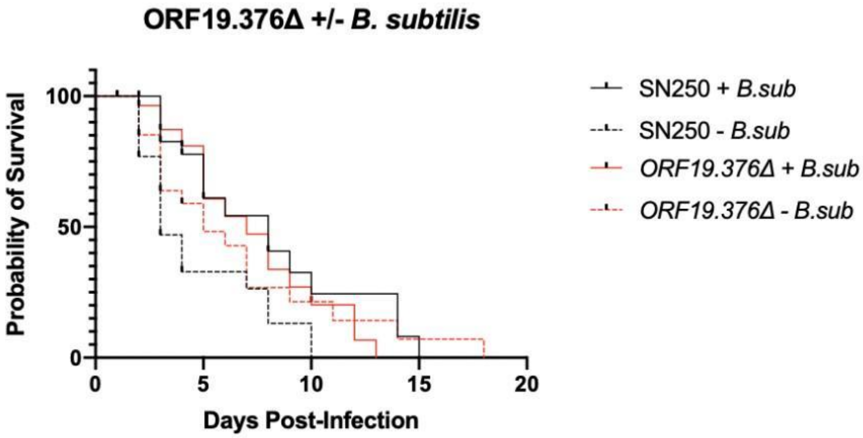


Figure 13: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.376*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *nuo2* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.376* knockout *C. albicans* mutant.

Table 12: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.376*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	ORF19.376Δ + B. sub.	ORF19.376Δ - B. sub.	SN250 + *B. sub.*	SN250 - *B. sub.*	ORF19.376Δ + B. sub.	ORF19.376Δ - B. sub.
	Gehan-Breslow-Wilcoxon test				LogRank			
SN250 + *B. sub.*		0.0011	NS	-		0.0026	NS	-
SN250 - *B. sub.*	0.0011		-	NS	0.0026		-	NS
ORF19.376Δ + B. sub.	NS	-		NS	NS	-		NS
ORF19.376Δ - B. sub.	-	NS	NS		-	NS	NS	

Gene: *ORF19.380Δ*

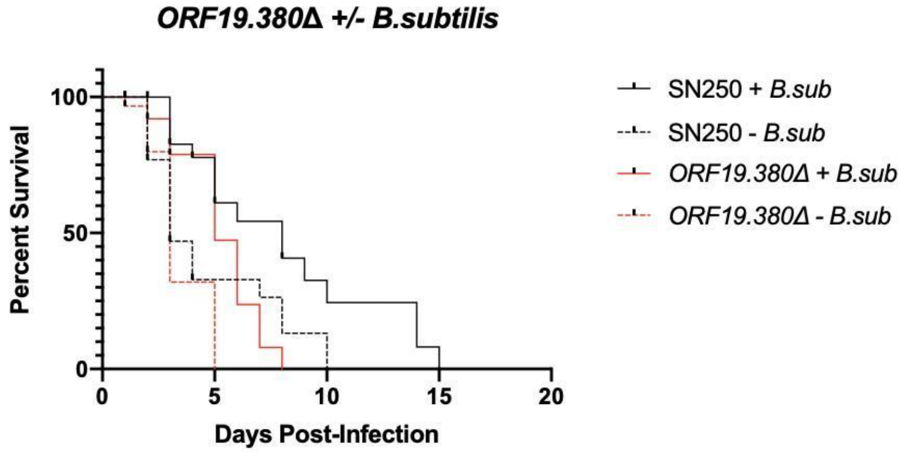


Figure 14: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *ORF19.380*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *nuo2* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *ORF19.380* knockout *C. albicans* mutant.

Table 13: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *ORF19.380*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.380*Δ + *B. sub.*	*ORF19.380*Δ - B. *sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*ORF19.380*Δ + *B. sub.*	*ORF19.380*Δ - *B. sub.*
	Gehan-Breslow-Wilcoxon test				LogRank			
SN250 + *B. sub.*		0.0011	NS	-		0.0026	0.0241	-
SN250 - *B. sub.*	0.0011		-	NS	0.0026		-	NS
*ORF19.380*Δ + *B. sub.*	NS	-		0.0014	0.0241	-		0.0002
*ORF19.380*Δ - *B. sub.*	-	NS	0.0014		-	NS	0.0002	

Gene: *iff8*

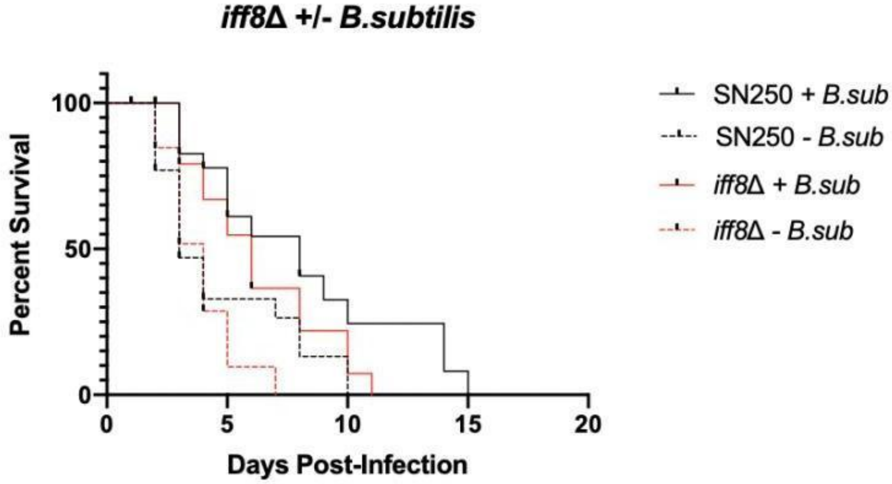


Figure 15: Survival Assay of *B. subtilis* treated and untreated wild type *Candida albicans* and mutant *iff8*Δ. The x-axis represents the day post-infection, and the y-axis represents the percent survival rate of the *C. elegans*. The black solid line is the control wild type *C. albicans* treated with *B. subtilis*. The dotted black line is the control wild type untreated *C. albicans*. The red solid line is *iff8* knockout *C. albicans* mutant treated with *B. subtilis*. The dotted red line is untreated *iff8* knockout *C. albicans* mutant.

Table 14: Statistical significance of Gehan-Breslow-Wilcoxon and Log-rank p-value in *C. elegans* survival assay of treated/untreated wild type *Candida albicans* and mutant *iff8*Δ. Values that had no significance are labeled NS. Comparisons that are not discussed have a dash.

	SN250 + *B. sub.*	SN250 - *B. sub.*	*iff8*Δ + *B. sub.*	*iff8*Δ - *B. sub.*	SN250 + *B. sub.*	SN250 - *B. sub.*	*iff8*Δ + B. sub.	*iff8*Δ - B. sub.
	Gehan-Breslow-Wilcoxon test				LogRank			
SN250 + *B. sub.*		0.0011	NS	-		0.0026	NS	-
SN250 - *B. sub.*	0.0011		-	NS	0.0026		-	NS
*iff8*Δ + *B. sub.*	NS	-		0.0047	NS	-		0.0016
*iff8*Δ - *B. sub.*	-	NS	0.0047		-	NS	0.0016	