Using Competition Assays to Assess Dormancy: Effects of Gene Knockouts on Growth in *Micrococcus luteus*

A Senior Honors Thesis Presented to

The Faculty of the Department of Biology and Biochemistry

University of Houston

In Partial Fulfillment

of the Requirements of the Degree

Bachelor of Science

By

Rene' Elizabeth Zimmerer

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Using Competition Assays to Assess Dormancy: Effects of Gene Knockouts on Growth in *Micrococcus luteus*

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ABSTRACT

Dormancy is a survival growth state some bacteria enter under stressful environmental conditions, such as nutrient deprivation. Dormancy is characterized by a severe decrease in metabolic activity where bacteria become viable but not culturable (VBNC), which is measurable by plating and counting colony forming units (CFU). This decrease in metabolic activity also reduces the effectiveness of antibiotics as they are less able to enter cells. For this reason, understanding the mechanism of initiating, sustaining, and resuscitating bacterial dormancy is critical for improving treatment of bacterial pathogens utilizing dormancy to evade antibiotic treatment.

Competition experiments can measure the relative fitness of bacterial populations by co-culturing the two (or more) populations and allowing them to compete for the same resources. My goal was to use a simple competition assay to measure the relative fitness of wild-type *Micrococcus luteus* to gene knockout strains of genes suspected in the mechanism of dormancy, such as the *uspA616* gene. First I demonstrated that the pigment synthesis gene, *crtE*, in *M. luteus* is a neutral site in both nutrient rich and nutrient poor media. Knockout of the *crtE* gene (*ΔcrtE*::kan) produced white bacterial colonies, as opposed to yellow colonies in wild-type *M. luteus*. The *ΔcrtE*::kan bacterial strain was found to have similar fitness to wild-type and was therefore used as an easily identifiable wild-type substitute in all other competition experiments. With the white *ΔcrtE*::kan *M. luteus* strain I then show that the UspA616 gene knockout (*ΔuspA616*::kan) strain has similar fitness to wild-type *M. luteus* in nutrient rich media, but is significantly less fit in nutrient poor media.

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CFU	colony forming units
G+C	guanine + cytosine
Kan	kanamycin, the insert used to inactivate genes
MAM	minimal acetate media
mNBE	modified Nutrient Broth E
NaCl	sodium chloride
NaOH	sodium hydroxide
OD/OD_{600}	optical density measured with a 600 nm wavelength
PBS	phosphate buffer saline
TB	Tuberculosis
WT	wild-type
ΔcrtE	*M. luteus* strain with an inactivation of the *crtE* gene
ΔuspA616	*M. luteus* strain with an inactivation of the *uspA616* gene

Chapter 1

Introduction and Background

The object of this senior honors project is to develop a plating assay for distinguishing phenotypes between wild-type *M. luteus* and specific gene knockouts that are involved in a dormant bacterial phenotype. *M. luteus* has a well-studied dormant phenotype. I have taken advantage of this reproducible phenotype to developed and test my hypothesis that the *ΔcrtE*::kan *M. luteus* mutant is a white neutral mutation and can act as a substitute for the yellow wild-type. This color selection was then used as a method to determine fitness of mutations that affect *M. luteus* ability to enter dormancy. This thesis is a study of growth and competition between these bacterial strains.

1.1 *Dormancy*

Bacteria grow rapidly when the surrounding environment is optimal, including abundant nutrients, however when nutrients become limited they must adapt. Bacteria can adapt to stress in many ways including sporulation, the stringent response, and dormancy. Each of these responses exhibit major remodeling of gene expression and protein products in an attempt to overcome their environmental conditions. Endospore formation follows a highly regulated developmental process that occurs in many Gram-positive bacteria. Other bacteria (like *E. coli*) can just stop growing in order to prolong stationary phase,

but this is a short term solution, and if the external stress is not removed, the cells will eventually die.

Dormancy is a survival state where some bacteria (like *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Treponema pallidum*) become viable but nonculturable (VBNC). In a VBNC state bacteria dramatically decrease metabolic activity, including respiration, and cease cell replication; however, VBNC cells can be resuscitated (initiate reproduction) once conditions become more favorable. Dormancy, in clinical terms referred to as latency, can be induced by nutrient deprivation, hypoxia, extreme pH, the host immune system, or the presence of antibiotics. Latency allows bacteria to remain VBNC for years (Greenblatt et al., 2004) (Heim et al., 2002). This mechanism of dormancy causes bacteria to have decreased sensitivity to a broad spectrum of antibiotic drugs.

One such example is Tuberculosis (TB), which is caused by the inhalation of airborne droplets containing *Mycobacterium tuberculosis* (*M. tuberculosis*). TB is the ninth leading cause of death worldwide. More than two billion individuals are estimated to be infected with *M. tuberculosis*. The World Health Organization reported approximately 10 million cases of infection and 1.3 million deaths from TB in 2017 (2018). The ability of *M. tuberculosis* to enter a dormant state means even antibiotic susceptible strains can become drug resistant and resuscitate at a later time causing an active infection from a latent infection.

Unfortunately, little is known about the mechanisms inducing, maintaining, and resuscitating bacterial dormant states. It is imperative to better understand this critical tool bacterium use to increase antibacterial tolerance. It is the stated goal of the World Health Organization to eradicate Tuberculosis; however, latent infections would make achieving this goal almost impossible (Gengenbacher and Kaufmann, 2012).

1.2 *Micrococcus luteus*

The molecular pathway(s) controlling dormancy are difficult to study due, in part, to limitations in current bacterial models used for studying dormancy, including *M. tuberculosis* and *Mycobacterium smegmatis* (*M. smegmatis*). Both *M. tuberculosis* and *M. smegmatis* are restrictive models because they exhibit very slow growth, with doubling times of 24 hours and 3 hours, respectively. Additionally they both have highly redundant genomes, making knockout studies difficult to engineer because of the need for multi-gene deletions. The model organism chosen here for studying bacterial dormancy was *Micrococcus luteus* NCTC 2665 (*M. luteus*). *M. luteus* is a non-pathogenic Gram-positive obligate aerobe that is found in soil, water, as part of the natural flora of mammalian skin and even inside the human body. *M. luteus* was first discovered by Sir Alexander Fleming and was used in his experiments on the study of lysozyme as a bacterial strain that was especially sensitive to the lytic properties of the enzyme (Fleming and Allison, 1922).

M. luteus is related to Mycobacteria; they are all from the phylum Actinobacteria. *M. luteus* has several advantages for studying dormancy, including non-pathogenicity, reproducible and separable growth stages that include dormancy, and a fast doubling time of 40 minutes. The *M. luteus* genome (NC_012803) is currently sequenced as a size of 2,501,097 base pairs, making it one of the smallest known prokaryotic genomes, smaller than both the *M. tuberculosis* H37Rv genome (NC_000962.3) and the *M. smegmatis* MC2 155 genome (NC_008596.1), which have genome sizes of ~4.4 Mbp and ~6.9 Mbp respectively. *M. luteus's* genome contains 32 pseudogenes and 2,230 protein sequences coding for 2,217 proteins and has a high G+C content of approximately 73%. Of significant importance, the genome of *M. luteus* has few protein redundancies and produces a protein that can induce a resuscitation pathway in all three bacteria, called Resuscitation Promoting Factor (Rpf) (Mukamolova et al., 1918). The ability of Rpf to cause revival out of dormancy to a culturable state in each bacterium suggests a similar mechanism of resuscitation between these and possibly other bacteria that are capable of entering dormancy.

1.3 *Universal Stress Proteins*

The universal stress proteins (Usp) are a superfamily of proteins found in bacteria, archaea, fungi, plants, and protozoa. Their name derives from the fact that these proteins are typically upregulated by environmental stresses. Universal stress proteins appear to have three main structural forms; proteins with a single

usp sequence domain, proteins with tandem *usp* domains and proteins with a *usp* domain fused with another functional domain (Nachin et al., 2008). While universal stress proteins are evolutionarily widely distributed; the exact physiological role of these proteins has yet to be elucidated (Kvint et al., 2003).

Universal stress proteins are of interest for studying dormancy because the three universal stress proteins, UspA616 (WP_010079616.1) found in *M. luteus*, were shown by mass spectroscopy to be upregulated in dormant state cells while absent in exponentially growing cells. A different universal stress protein, UspA712 (WP_10079712.1) was upregulated in exponentially growing cells, while not observed in dormant cells (Mali et al., 2017). Both of these proteins were named as a reference to the last few digits of their accession numbers. The universal stress protein WP_010079616.1 contains two Usp domains. Knockout mutants each of the three *usp* genes have been generated in *M. luteus*, and in this thesis, I show the relative fitness of the *uspA616* knockout.

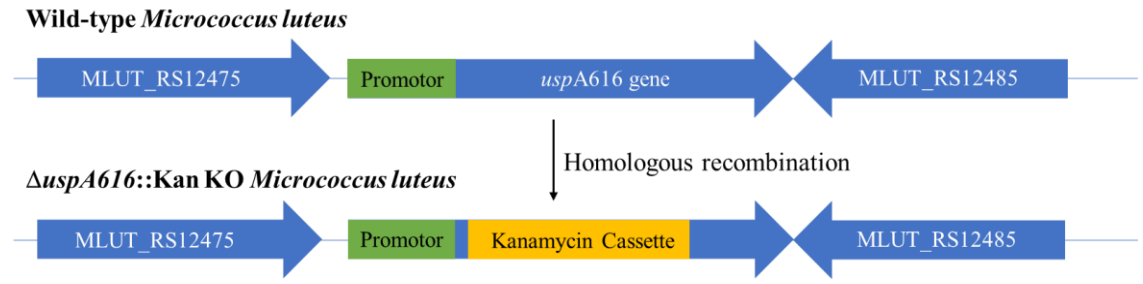

Figure 1.1: Generation of *uspA616* Gene Knockout in *Micrococcus luteus*

Knockout of the *uspA616* gene in the *Micrococcus luteus* by insertion of a Kanamycin cartridge through homologous recombination. The *ΔuspA616*::kan strain was produced by graduate student, Spencer Havis.

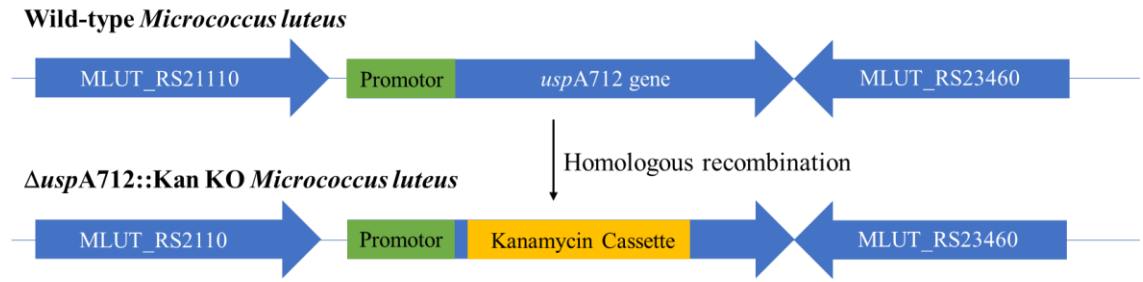

Figure 1.2: Generation of *uspA712* Gene Knockout in *Micrococcus luteus*

Knockout of the *uspA712* gene in *Micrococcus luteus* by insertion of a Kanamycin cartridge through homologous recombination. The *ΔuspA712*::kan strain was produced by graduate student, Abi Bodunrin.

1.4 *Individual Growth Curves*

Growth of a bacterial population can be quantified by inoculating fresh medium with a given number of cells and monitoring this culture over time. Bacterial enumeration at different times can be accomplished by removing samples from the growth culture, diluting in saline solution, then plating the dilution for colony counting.

The goal of the growth curve experiments was to test the hypothesis that a knockout in the *crtE* gene would not affect the reproductive fitness of the bacteria, and that the *⊿crtE*::kan strain would grow in an identical manner to wild-type *M. luteus* whether in a carbon rich (low stress) environment, or in a carbon poor (starvation stress) environment.

1.5 *Competition Assays*

Targeted gene inactivation, commonly known as gene knockout enables scientists to study the functional importance of specific genes and the impact of specific genetic deletions on complex metabolic processes. However, quantifying the results of a gene knockout must be accomplished by comparing the phenotype of the mutant to the wild-type organism. For bacteria, competition experiments allow the comparison of relative fitness between mutant and wild-type strains.

Competition assays are experiments in which two bacterial strains are grown together in a co-culture and therefore compete for the same resources. The

relative fitness of the two populations can be measured by calculating the natural logarithm of the ratio of cell densities over time. Typically, the ratio of cell densities is estimated from the number of colony forming units (CFU) of a diluted co-culture sample.

To run competition experiments, a difference in appearance between the two co-cultured strains is required to allow identification and counting of each population's CFU numbers. Wild-type *M. luteus* has a carotenoid producing operon containing seven genes coding for the proteins needed to modify Farnesyl pyrophosphate into the sarcinaxanthin carotenoid which cause *M. luteus* colonies to be a yellow color. To differentiate strains in competition experiments the first gene of the carotenoid operon (*crtE*) was knocked out with a kanamycin cartridge by homologous recombination creating a *ΔcrtE*::kan *M. luteus* strain (Figure 1.3). This construct was created by Zina Housammy in Dr. Widger's laboratory. In wild-type *M. luteus*, the CrtE enzyme is responsible for converting Farnesyl pyrophosphate into Geranylgeranyl pyrophosphate, however this step and therefore sarcinaxanthin synthesis is terminated in *ΔcrtE*::kan *M. luteus*. As a result, *ΔcrtE*::kan *M. luteus* forms white colonies (see Figure 1.4).

The *crtE* gene was hypothesized to be a neutral gene, and therefore a mutation of that site was expected to have no effect on the fitness of *ΔcrtE*::kan *M. luteus* relative to wild-type *M. luteus*. This hypothesis was tested, results and further discussion are presented in Chapters 3 and 4.

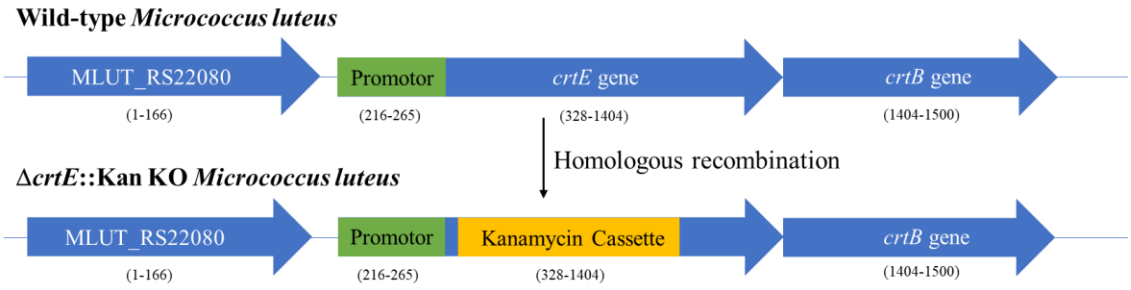

Figure 1.3: Generation of *crtE* Gene Knockout in *Micrococcus luteus*

Knockout of the first gene in the *Micrococcus luteus* sarcinaxanthin operon, *crtE*. This produced a white kanamycin resistant phenotype in this *M. luteus* strain. The *ΔcrtE*::kan strain was produced by undergraduate student, Zina Housammy.

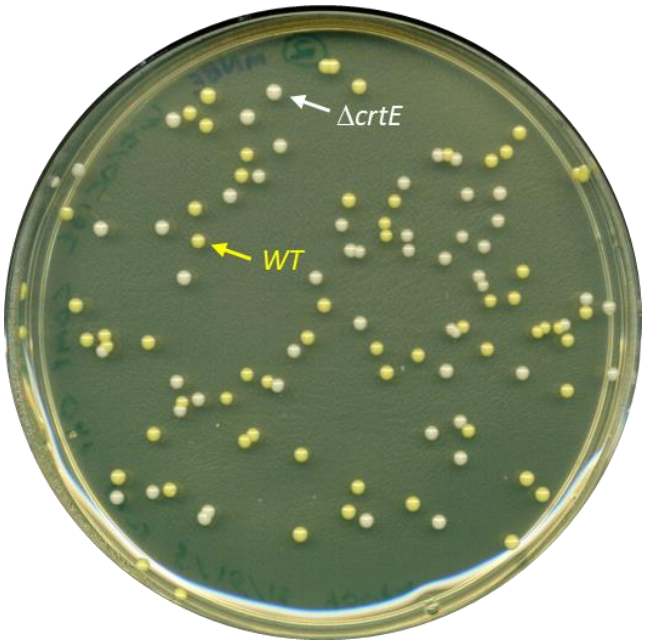

Figure 1.4: Plate with Colony Forming Units from Co-culturing Wild-type and *ΔcrtE*::kan *Micrococcus luteus*

Yellow wild-type (WT) and white *ΔcrtE*::kan *M. luteus* colonies plated on a nutrient rich (mNBE [modified nutrient broth E] agar plate. This plate was made by plating a diluted sample from a *ΔcrtE* vs WT competition co-culture flask.

Chapter 2

Materials and Methods

2.1 *Materials*

All chemicals and reagents used were obtained from Fisher Scientific, Bio Rad, and/or Sigma Chemical Company. Disposable pipette tips were obtained from Fisher and/or Eppendorf. Pipettors were obtained from VWR and Gilson. Lab machinery includes a Cary Varian 50 spectrophotometer and an Epson Perfection 2580 photo scanner.

2.2 *Bacterial Stocks*

All *M. luteus* stocks were grown from the same strain of *M. luteus*; NCTC 2665 “Fleming strain” obtained from the American Type Cell Culture company (cat # 4698). Cells were stored as glycerol stocks at -80 °C.

2.3 *Preparing Growth Media*

One liter of rich liquid media was made by adding 5 g Tryptone, 5 g NaCl, and 3 g yeast extract and approximately 500 mL of DI H_2O to a 1-liter beaker. The mixture was stirred using a magnetic stir bar until dissolved and pH was adjusted to 7.4. The mixture was then poured into a one-liter graduated cylinder and DI H_2O was added until the total volume was one liter. For larger or smaller volumes, a beaker size of twice the added volume was used to prevent spill-over while stirring. The media was then aliquoted into the desired containers, covered and then autoclaved to avoid contamination. Starter tubes contained 5 mL of rich

media and were capped with metal or plastic tops. Rich media co-culture flasks contained 50 mL of media and were covered in twice folded (four layers) aluminum foil squares that were tightly wrapped around the neck of each flask. The autoclave heated the media to 121 °C for 20 minutes.

Making one liter of rich media agar required the addition of 15 g of agarose directly after the rich media was prepared as listed above. The agar media was then covered and autoclaved for 20 minutes at 121 °C. Agar media can be poured into plates immediately after removal from the autoclave, before it solidifies at room temperature. Rich media agar was made for later use with 500 mL of rich media was aliquoted into 1-liter bottles then 7.5 g of agar was added to each bottle. The plastic caps were screwed on loosely before the bottles were heated in the autoclave. After autoclaving, the caps were screwed on tightly to keep out contaminants. When rich media plates needed to be poured the agar bottle was placed in the microwave with the cap screwed on loosely to allow air to escape the container. The agar was microwaved until completely liquid with intermittent stirring every two minutes. Once all the agar melted it was cooled slowly on the benchtop until it was cool enough to pour into plates.

To make a kanamycin agar plate, 100 mL autoclaved liquid agar was mixed with 100 µL of 100 mg/ml kanamycin to a final concentration of 100 μg/ml just before pouring the plate. If other volumes of kanamycin agar were required, a ratio of 1,000:1 agar to antibiotic was made.

Preparing 500 mL of liquid minimal acetate media required 300 mL of DI H_2O to be mixed with 50 mL of a 10X Minimal Media Salt stock solution (40 g/literNH_4Cl, 14 g/liter K_2HPO_4 at pH of 7.4), 7.48 g of sodium acetate trihydrate ($NaOAc•3H_2O$), 0.5 g inosine, 5 mL of a 100X trace metal stock solution (14.3 g/liter $MgSO_4•7H_2O$, 0.00375 g/liter $CuSO_4•5H_2O$, 0.079 g/liter $MnCl_2•4H_2O$, 0.183 g/liter $FeSO_4•7H_2O$, 0.025 g/liter Na_2MoO_4, and 0.005 g/liter $ZnSO_4•7H_2O$), 0.5 g yeast extract, 25 mL of 1 M HEPES buffer at pH of 7.4 (which will cause the final minimal acetate solution to have a 50 mM HEPES concentration). The final volume was brought up to 500 mL with DI H_2O. This solution was distributed into desired flask sizes, capped with aluminum foil, then autoclaved. After sterilization, 1,000X vitamin supplement stocks of 20 μg/ml Methionine, 5 μg/ml Biotin, and, 40 μg/ml Thiamine were added to each flask. Ratios of vitamins were: 0.01 mL Methionine, 0.02 mL Biotin, and 0.01 mL of Thiamine for every 10 mL of Minimal Acetate solution.

2.4 *Plating Micrococcus luteus*

The source of bacteria for all experiments were glycerol stocks of the NCTN 2665 Fleming strain (wild-type and Δ*crtE*::kan) of *M. luteus* stored at -80 °C in Eppendorf tubes. To plate *M. luteus*, both the freezer stock and a 10 cm agar plate were placed in the biosafety cabinet. Depending on which *M. luteus* strain being plated, either a rich media plate (for wild-type) or a kanamycin rich media plate (for mutant knockouts) was used. An inoculation loop was heated over a flame until bright red. While hot, the loop was pushed into the frozen cell stock

which thawed the bacteria in contact with the loop. The loop was then streaked lightly and widely across the plate into four or five sections. The freezer stocks were immediately returned to the -80 °C freezer. The streaked plate was labeled on the bottom of the plate with the strain of *M. luteus*, type of agar media, the date of streaking, and my initials before being placed in a 30 °C incubator for approximately three days when individual colonies were large enough to pick.

2.5 *Inoculation of Starter Cultures*

The tubes of 5 mL sterilized rich media were labeled with the name of bacterial strain, date and time of inoculation, type of media, and my initials. The tubes and the agar plate containing individual colonies of the *M. luteus* strain needed to make the starter culture were placed in the biosafety cabinet. The plate contained colonies originating from the freezer stocks and was viable to use for starter cultures if no contamination occurred on the plate. A container of autoclaved toothpicks was unscrewed. Metal tweezers were heated with a Bunsen burner flame until hot. Using the tweezers, one toothpick was carefully grabbed from the container. While holding the toothpick in the tweezers, a single colony was removed using the tip of the toothpick. The agar plate was covered immediately after a colony was removed to prevent contamination. The cap of the starter culture tube was removed, and the end of the tube was rotated over the flame for five seconds. The toothpick was carefully dropped into the media, finally the end of the tube was flamed again before placing the cap back on the tube. These steps were repeated for as many starter cultures that were needed.

For all experiments, at least two starter cultures for each *M. luteus* strain were made in case one failed to grow or contamination occurred. Starter tubes were grown in shakers operating at 300 rpm at 30 °C overnight. Both carbon rich, modified nutrient broth E (mNBE), and carbon deficient minimal acetate media (MAM), co-culture competition flasks were inoculated from rich media overnight starter cultures. The ability to grow cells in flasks reduced significantly if overnight starter cultures were incubated in the shaker for more than twenty hours.

2.6 *Glass Bead Sterilization*

Glass beads were used to evenly distribute diluted sample of the co-culture across an agar plate. The glass beads were sterilized by soaking in bleach for at least twenty minutes. Then washed with warm soap and water, then thoroughly rinsed to remove any bleach and soap residue. The glass beads were placed in a glass tube and covered with aluminum foil before being autoclaved. After the autoclave the glass tube was placed in a 256 °C oven until all water was evaporated from the glass beads. The glass tube was removed from the oven and allowed to cool before storing in the biosafety hood for use during plating.

2.7 *Absorption Spectroscopy*

Absorbance spectroscopy was used to measure the cell density of the cultures using a Cary Varian 50 spectrophotometer and 600 nm using 1 mL quartz cuvettes. Cell samples were measured directly unless the OD_{600} was over 1.0. Any culture samples measuring over 1.0 OD/mL were diluted 1 to 10 by adding

0.1 mL of cell sample in 0.9 mL of either fresh media, for starter tube samples or Phosphate Buffer Saline (PBS), for co-culture competition samples. To minimize reading disruptions, the sides of each cuvette was cleaned with a Kim Wipe to remove fingerprints or liquid droplets before placement into the sample compartment. The spectrophotometer was blanked using either 1 mL of fresh media (for starter tube samples) or PBS (for competition samples) and the optical density was recorded. Following the blank solution, sample cuvettes were loaded, and the optical density was recorded. Samples from starter tubes were removed for OD readings after overnight incubation, just before inoculation of competition co-culture flasks. Samples from the co-culture flasks were taken every 12 hours (for nutrient rich media experiments) or every 24 or 48 h (for nutrient poor media experiments).

2.8 *Serial Dilutions*

The stepwise process of serial dilution was used to decrease the concentration of cells from the co-culture flasks to allow distinguishable colony forming units (CFUs) to form on agar plates. Serial dilutions required many 1.7 mL Eppendorf tubes containing 450 µL of PBS. To prepare these dilution tubes, a pipette was used to aliquot 450 µL of PBS into Eppendorf tubes. These tubes were then capped, placed on a rack, then wrapped in aluminum foil and placed in the autoclave. After sterilization, the rack was placed in the biosafety cabinet for later use in experiments. To make serial dilutions a pipettor was used to remove 50 µL from a co-culture flask to put into one of the Eppendorf tubes containing 450 µL

PBS (1 to 10 dilution). The dilution was mixed by slowly pipetting the solution up and down. 50 µL of this solution was then removed with the same pipet tip and added to the next Eppendorf tube. This process was repeated until the desired dilution was reached. Typically, dilutions between 10^{-3} to 10^{-8} were made.

2.9 *Individual Growth Curve Experiments*

Fresh colonies of wild-type and *⊿crtE*::kan *M. luteus* were streaked on plates. Wild-type was streaked on a rich media (mNBE) plate, while *⊿crtE*::kan was streaked on a kanamycin rich media plate. These plates were incubated at 30 °C for approximately three days, at which time individual colonies were large enough to pick. Fresh starter cultures were inoculated from some of the colonies on each plate. The starters were grown at 30 °C overnight at 300 rpm. For rich media experiments, 0.5 mL of starter culture volume was inoculated into 50 mL of liquid mNBE. For nutrient poor media experiments, 0.5 mL of starter culture volume was inoculated into 100 mL of liquid minimal acetate media (MAM). The previous step was repeated three times, to create three biological replicates (from the same starter). The three biological replicate cultures were covered and incubated aerobically in a 300 rpm shaker at 30 °C. OD_{600} and CFU measurements were taken every twelve h for a total of three days (for nutrient rich experiments) and measurements were taken every 24 or 48 h for a total of seven days (for nutrient poor experiments).

For growth curve experiments, CFUs were generated using a drop plate technique in which after the samples were diluted, 10 µL drops of the desired

dilution was carefully placed on the mNBE agar plate. A total of fifteen 10 µL drops were placed around the plate, making sure not to have two drops contact each other. After the plate was covered in drops the plate was allowed to sit undisturbed for a few minutes to let the agar absorb the liquid in each drop so the plates could be covered, flipped, and placed in the 30 °C incubator. The plates stayed in the incubator for 36-48 h, or until individual colonies were just large enough to be counted. At this time the plates were scanned with the Epson Perfection 2580 photo scanner for later data collection (CFU counting). The CFU data collected for growth curve experiments is presented in the Appendix.

2.10 *Competition Experiments*

Fresh colonies of the two strains to be used in the co-culture competition experiment were streaked on plates. Wild-type *M. luteus* was streaked on rich media (mNBE) plates. *ΔcrtE*::kan, *and ΔuspA616*::kan strains were plated on kanamycin rich media (mNBE) plates. The plates were incubated in a 30 °C incubating room for approximately three days, or until colonies were large enough to pick for inoculation. Starter tubes were placed on a 300 rpm shaker at 30 °C overnight. This allowed cultures to grow to approximately 1.0 OD_{600}/mL, indicating the population was in its exponential growth phase. The cell density (OD_{600}/mL) of each starter was measured by the Cary Varian 50 spectrophotometer, and the two starter cultures with the optical density values closest each other were chosen for use in inoculating the competition co-culture flasks. The formula: $OD_1V_1 = OD_2V_2$ was used to adjust the volume so the

standard value of 0.15 OD of cells from the starter tube was added to the co-culture flask. Where OD_1 is the OD of the starter culture grown overnight, V_1 is the volume of this starter culture to be added to the co-culture flask, OD_2 is the standard OD of 0.15, and V_2 is the co-culture volume. The same process was used to calculate the second starter tube volume inoculated into the same co-culture flask. Once the volume of both starters was calculated, these volumes were added to each of the biological replicates.

The zero hour was the time of co-culture inoculation by the two starter culture strains. Four media containing flasks were labeled with the experiment title, date, and time of inoculation, growth media used, my initials, and a number (1, 2, 3, or 4) as a designation of which biological replicate that flask would represent. For nutrient rich media experiments, the inoculum was put into 250 mL flasks containing 50 mL liquid mNBE, and for nutrient poor media experiments, the inoculum was put into 250 mL flasks containing 100 mL of liquid MAM. Once the four labeled flasks, and the two starters were placed in the biosafety cabinet, the cap of one starter tubes was removed, and the open end of the tube was rotated over a flame for 1-2 ss any time a cap was removed and just before recovering the tube. The aluminum foil cover was removed from one of the conical flasks, the lip of the flask and the aluminum foil cover was flamed for 1-3 ss any time the cover was removed and just before replacing the aluminum cover on the flask. A pipet was used to remove the desired volume from the starter and added to the open media flasks. This was repeated for each of the four media flasks. This

process was repeated with the other starter tube until all four flasks (now called co-cultures and biological replicates) had both *M. luteus* strains. Once cool enough to hold, the flasks were placed on a 300 rpm shaker in a 30 °C incubating room.

For nutrient rich media experiments, samples were taken from the co-cultures every 12 h for a total of three days. For nutrient poor media experiments, samples were taken every 24 h for at least seven days.

Before a sample was removed from the four co-cultures for plating CFUs, optical density measurements were collected to help estimate cell concentration of each flask. Knowing cell concentration allowed estimation of the dilution needed to plate the optimum number of cells for CFU counting. Once the co-culture flasks and cuvettes were brought to the biosafety cabinet the aluminum foil cover was removed from one flask. The flask was swirled to disperse any cells that settled on the bottom of the flask and a sample was carefully removed from the culture and aliquoted into a cuvette. The flask neck and the aluminum cover were sterilized by holding them over the Bunsen burner flame before the aluminum cover was wrapped back around its flask. This process was repeated for each of the biological replicates. After setting the spectrophotometer to a 600 nm wavelength, a cuvette containing 1 mL of PBS was used to zero the spectrophotometer followed by each of the four cell sample cuvettes.

After acquiring cell density information, serial dilutions were made from each of the co-cultures. Typically, when the cultures had an OD less than 0.4, the

optimum dilutions were 10^{-3} or 10^{-4}. For optical densities between 0.4 and 3 OD/mL the best dilutions were 10^{-5} or 10^{-6}. For optical densities above 3 OD, dilutions of 10^{-7} or 10^{-8} were optimal. To plate a serial dilution sample, the agar plate cover was removed, and a pipette was used to aliquot 100 µL from a chosen dilution the mNBE agar plate. This step was repeated two more times with two different agar plates, so three plates (technical triplicate) were made for each dilution. Once all three plates had 100 µL of sample, about 10 glass beads were poured onto each plate. The covers were placed back on the plates and the plates were gently swirled causing the beads to distribute the sample across the entire plate. The plate covers were removed temporarily to allow the glass beads to be dumped into a beaker of “used” beads. Two serial dilution samples were chosen to plate for all the co-cultures because this would allow me to choose the dilution that had better CFU data.

Once all the co-cultures had two dilutions plated in technical triplicate (a total of 24 plates) the plates were incubated at 30 °C for approximately three days, or until individual colonies and the color of the colonies could be distinguished. The co-culture flasks were placed back in the 300 rpm shaker at 30 °C until the next sampling time. After approximately 3-4 days of incubating the plates had individual colonies of distinguishable color. The plates of the best dilution were chosen for scanning to collect CFU data. The best plates were chosen based on if they had (usually) more than n = 30 colonies, but not so many colonies that

colony separation was prohibited. The CFU data from competition experiments is presented in the appendix.

2.11 *Counting CFUs and Data Analysis*

To collect CFU data, each plate was scanned using an Epson Perfection 2580 photo scanner with a black background to enhance visualization of the color of each colony. The images were saved as tiff files and later processed using ImageJ (https://imagej.nih.gov/ij/index.html). For the individual growth curve experiments the images were processed by first converting the tiff file to 16-bit and threshold was adjusted to filter colonies from the background. The watershed binary was used to separate colonies that were very close to each other. The Analyze Particle Size was set from 5 to infinity in pixel units to select colonies rather than background noise.

For the competition experiments, the image processing described above was not an option because ImageJ is not able to distinguish the color differences between white and yellow colonies on each plate, and therefore was not able to separate white vs yellow CFU numbers. For this reason, the CFU counting for competition experiments was completed through manual counting of yellow and white colonies.

For both the individual and competition experiments, the number of colonies was averaged then normalized for dilution, and back-calculated to CFU/mL as well as the Log_{10} (CFU/mL) for easier statistical comparisons and growth curve figures.

Chapter 3

Growth curves

3.1 *Introduction and Background*

Bacterial replication occurs by binary fission where a single bacterium divides into two genetically identical daughter cells. Growth of a bacterial population can be quantified by inoculating fresh medium with a given number of cells and monitoring this culture over time. Bacterial enumeration can be accomplished by various methods such as flow cytometry, colony counting, or measuring turbidity by absorption spectroscopy. The typical bacterial growth curve from a batch culture (a closed system – like a test tube), usually exhibit three different phases: the lag phase, the log or exponential phase, and the stationary phase.

Once cells are inoculated into a batch culture, they must adjust to their new environment. At this time the bacterial population is not experiencing cell division and this stage is termed the lag phase. Once bacteria have adjusted to the growth medium, the log or exponential phase begins. This is a period during which most of the cells in the population are dividing regularly and therefore the number of new bacteria appearing per unit time is proportional to the present population. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the growth rate of the organism under these specific growing conditions. Growth rate for bacteria is defined as the number of divisions per cell per unit time. Eventually in a batch culture, the rate of divisions declines, often due to the depletion of a

nutrient. At this time, the population transitions into stationary phase, where bacteria experience changes in metabolic activity in attempt to adapt to nutrient depletion. For some bacterial strains, if the culture is continually exposed to an environment decreasing in nutrients, the cells will eventually die. However, some cells, such as *M. luteus*, adapt to nutrient starvation by going latent. Latency (dormancy) is defined as a condition of viable but not culturable (VBNC) where the cells do not grow on solid media and require resuscitation to start growing. This process is distinct from sporulation, because latent cells are intact but have limited metabolic activity such as a loss in oxidative membrane potentials.

The goal of these growth curve experiments was to test the hypothesis that a knockout in the *crtE* gene would not affect the reproductive fitness of the bacteria, and that the *ΔcrtE*::kan strain would grow in an identical manner to wild-type *M. luteus* whether in a carbon rich or carbon poor environment. It was also important to execute growth curves in nutrient poor media because previous studies show that *M. luteus* grown in liquid minimal acetate media reproducibility entered the dormant phase upon nutrient starvation. All the lab studies on dormancy require growth on minimal acetate media, and therefore *ΔcrtE*::kan should exhibit similar growth to wild-type in MAM as well as mNBE media.

Growth curves where produced for wild-type and *ΔcrtE*::kan *M. luteus* in both carbon rich media (liquid mNBE) and carbon poor media (liquid MAM). Carrying out individual growth curve experiments (experiments in which only a single bacterial strain is in the batch culture) acts as a control for competition

experiments because it allowed for the quantification of bacterial growth without interference or cross-talk from the other bacterial strains (which may occur between co-culture competition experiments).

3.2 *Results*

3.2.1 Wild-Type and *ΔcrtE*::kan Individual Growth Curves in Nutrient Rich (mNBE) Media

The growth curves for both wild-type and *ΔcrtE*::kan *M. luteus* in liquid mNBE media were performed by removing batch culture samples every twelve hours for a total of three days and later counting CFU numbers. The CFUs were obtained by plating fifteen 10 µL dots (fifteen technical replicates) for each biological replicate. The OD_{600} and CFU numbers recorded for the three wild-type biological replicates and the same information for the three *ΔcrtE*::kan biological replicates is presented in the appendix as Tables A1-A3, and Tables A7-A9 respectively. To analyze the data the fifteen technical replicates of each biological replicate were divided into three arbitrary groups (five technical replicates in each group) and the average CFU for each group was calculated. The CFU/mL of each group was then back-calculated using the average CFU for each group and the serial dilution used to plate culture samples. The equation for calculating the CFU/mL is:

CFU/mL = ((CFU number) x (10^ dilution) / (volume plated in mL))

The Log_{10} (CFU/mL) for each group was calculated, then the average Log_{10} (CFU/mL) from all three groups was calculated. The Tables A4-6 in the appendix

shows the average CFU, CFU/mL, Log_{10} (CFU/mL), for each of the three groups as well as the average Log_{10} (CFU/mL) for all WT biological replicate. The same information is presented for the *ΔcrtE*::kan biological replicates in Tables A10-A12 of the appendix. The average and standard deviation of the average Log_{10} (CFU/mL) for all biological replicates was then calculated and is presented in Table 3.1. This data is also presented in graphical form in Figure 3.1.

Table 3.1: Average and Standard Deviation Log_{10} (CFU/mL) Values for Wild-type and *ΔcrtE*::kan *M. luteus* During Isolated Growth in mNBE Media

Time (Days)	Avg Log_{10} (CFU/mL) of WT Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) WT Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.
0	6.638228981	6.51480411	0.075951281	0.054045043
0.5	8.95462858	8.862927389	0.118695289	0.248332346
1	9.218110585	9.298854389	0.088388758	0.147327228
1.5	9.123333817	9.167966384	0.071564788	0.141909131
2	9.18026696	9.241250144	0.060485444	0.01898672
2.5	9.350381825	9.515497416	0.204562806	0.212615295
3	9.116616452	9.452355366	0.158161197	0.06429357

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the three biological replicates at each sampling time for both the wild-type and Δ*crt*E::kan batch culture in liquid mNBE media. This data was calculated from the raw data in Tables A1-A3 and A7-A9 (appendix) for wild-type and *ΔcrtE::*kan *M. luteus* respectively.

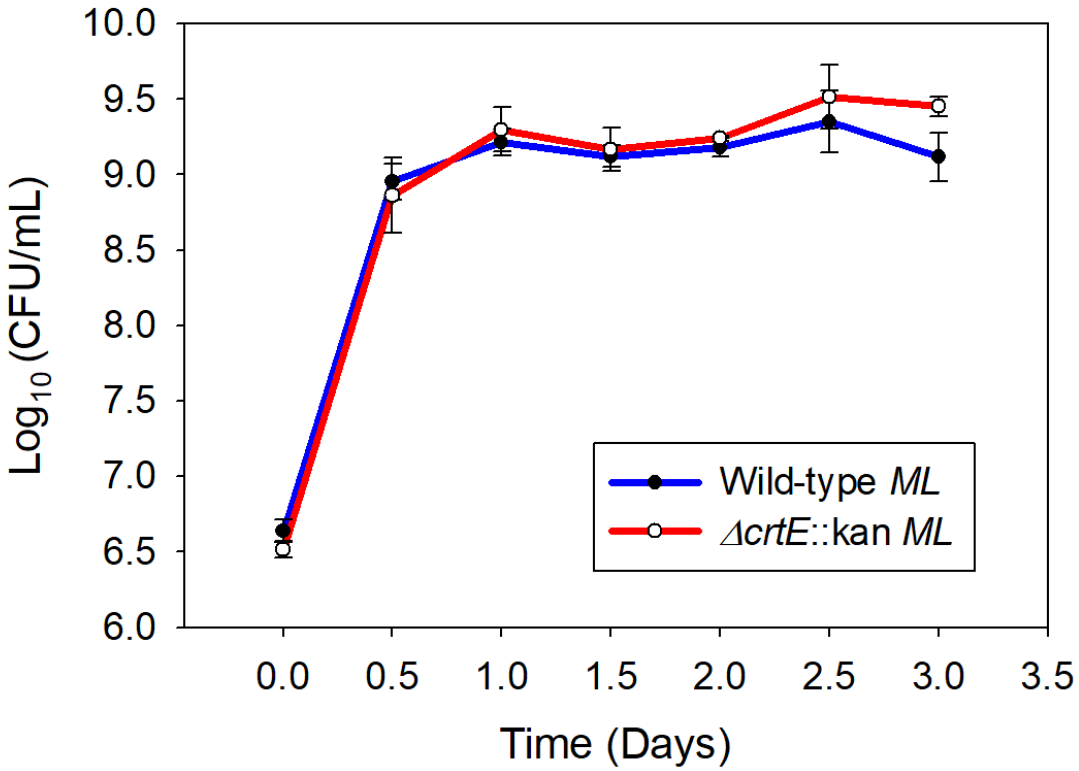

Figure 3.1: Isolated Growth Curves for Wild-type and *ΔcrtE::*kan *M. luteus* Cultured in Liquid mNBE Media

This figure graphically depicts the information presented in Table 3.1. Both the wild-type and *ΔcrtE*::kan *M. luteus* strains show very similar growth patterns in nutrient rich (mNBE) media.

3.2.2 Wild-Type and *ΔcrtE*::kan Individual Growth Curve in Nutrient Poor (MAM) Media

The growth curves for both wild-type and *ΔcrtE*::kan *M. luteus* in MAM media were performed by removing, diluting, and plating culture samples every 24-48 h for a total of seven days. The CFUs were sampled by plating fifteen 10 µL dots (fifteen technical replicates) for each biological replicate every 24-48 h. The OD_{600} and CFU data recorded for the three wild-type biological replicates and the same information for the three *ΔcrtE::*kan biological replicates is presented in the appendix as Tables A13-A15, and tables A19-A21, respectively. To analyze the data the fifteen technical replicates for each biological replicate, the fifteen data points were divided into three arbitrary groups (five technical replicates in each group) and the average CFU for each group was calculated. The CFU/mL of each group was then back-calculated using the average CFU for each group and the serial dilution used to plate culture samples. The Log_{10} (CFU/mL) for each group was calculated, then the average Log_{10} (CFU/mL) from these three groups was calculated.

Tables A13-A16 in the appendix shows the average CFU, CFU/mL, Log_{10} (CFU/mL), for each of the three groups as well as the average Log_{10} (CFU/mL) for each WT biological replicate grown in liquid MAM. The same information is presented for the *ΔcrtE*::kan biological replicates in Tables A19-A21 of the appendix. The average and standard deviation of the average Log_{10} (CFU/mL)

for all biological replicates at each sampling time was then calculated and is presented in Table 3.2.

Table 3.2: Average and Standard Deviation Log_{10} (CFU/mL) Values for Wild-type and *ΔcrtE*::kan *M. luteus* During Isolated Growth in MAM Media

Time (Days)	Avg Log_{10} (CFU/mL) of WT Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) WT Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.
0	6.638277719	5.698360112	0.04892975	0.092603551
1	9.33127705	9.088576413	0.033776806	0.068650429
2	8.847049696	8.606269247	0.062190415	0.06120394
3	8.72007236	8.561182938	0.097398561	0.218837829
5	7.488071305	6.887644993	0.40825978	0.139549274
7	7.212607134	6.366871213	0.091102885	0.093739266

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the three biological replicates at each sampling time for both the wild-type and Δ*crt*E::kan batch cultures in liquid MAM media. This data was calculated from the raw data in Tables A13-A15 and A19-A21 (appendix) for wild-type and *ΔcrtE::*kan *M. luteus,* respectively.

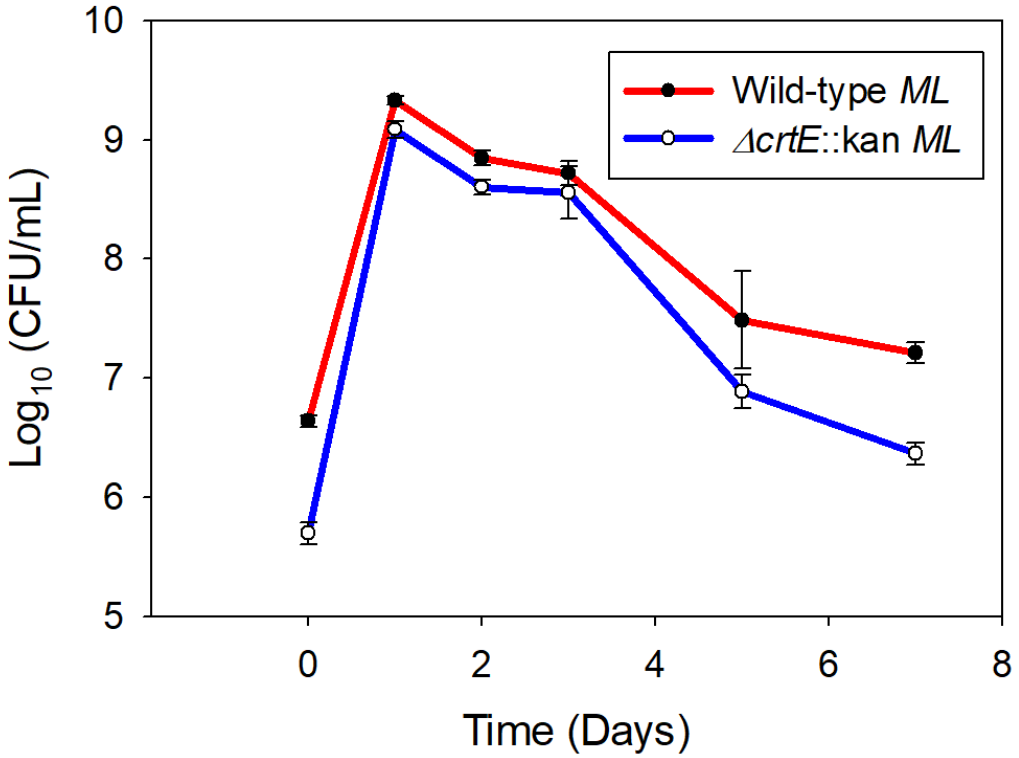

Figure 3.2: Isolated Growth Curves for Wild-type and *ΔcrtE::*kan *M. luteus* Cultured in Liquid MAM media.

This figure graphically depicts the information presented in Table 3.2. Both the wild-type and *ΔcrtE*::kan *M. luteus* strains show similar growth patterns in nutrient poor (MAM) media, however the wild-type strain consistently displays higher cell number over the seven-day experiment.

3.3 *Discussion and Conclusion*

The growth curve data supports the hypothesis stating the *crtE* gene is a neutral site and therefore knockout of this gene will not affect the mechanism of growth and dormancy in the *ΔcrtE*::kan mutant. The growth curve measurements for wild-type and *ΔcrtE*::kan *M. luteus* show the dividing ability of both strains over time. Comparing the growth curve data for both strains show there is no difference in growth phases between WT and *ΔcrtE*::kan strains in nutrient rich media (Figure 3.1) and a slight growth advantage for WT in nutrient poor media (Figure 3.2). In nutrient poor media, both WT and *ΔcrtE*::kan M. luteus experienced decreased CFU numbers after twenty-four hours and exhibit similar growth trends over time. This indicates both strains transition into a latency at the same time, and therefore suggests the stress response is unchanged when the *crtE* gene is inactive. Showing the *crtE* gene is a neutral site is important for allowing the *ΔcrtE::*kan mutant to act as a substitute for wild-type in competition experiments (discussed in Chapter 4) and as an insertion site for future gene rescue experiments.

Chapter 4

Competition Experiments

4.1 *Introduction and Background*

Understanding dormancy will ultimately depend on being able to identify the genes and proteins involved in the regulation of initiation and resuscitation of dormant phase cells. Gene knockout mutants are especially valuable for complex processes like dormancy because they demonstrate the impact a mutation or ablation in the gene of interest has on the organism, and therefore illustrate the functional importance of those genes. However, once a knockout mutant has been produced, a means to quantify the effect this ablation has on the organism relative to wild-type must be devised.

This thesis presents competition assays as a means of rapidly identifying the relative growth (fitness) of knockout mutants and wild-type *M. luteus*. Previous mass spectroscopy has allowed the identification of 18 proteins of interest which are not upregulated in exponentially growing *M. luteus* but are significantly upregulated in dormant state cells. One of these 18 proteins is a universal stress protein. Because universal stress proteins are involved in stress responses for many organisms, the three universal stress proteins in *M. luteus* are of interest in studying dormancy. Homologous recombination was used to produce knockouts of the three universal stress protein genes, *uspA616*, *uspA712*, and *uspA184*. Competition experiments have been completed for the *uspA616* knockout, and

competition experiments for the *uspA712* and *uspA184* knockouts are currently ongoing.

Unfortunately, competition experiments cannot be carried out with the UspA mutants and wild-type *M. luteus* because once in a co-culture together, there will be no way to identify the different colonies as they both produce yellow colonies. To resolve this issue the white mutant, *⊿crtE::*kan was produced. The ability to distinguish between white and yellow colonies, allows rapid assessment of CFU numbers for each strain in a co-culture, however relating the CFU numbers of the UspA mutants back to CFU numbers of wild-type *M. luteus* would only be possible if the white *⊿crtE::*kan strain grows identically to wild-type *M. luteus* in both mNBE and MAM. The growth curves (Chapter 3) showed the ability of *⊿crtE*::kan to grow similarly to wild-type *M. luteus* in both mNBE and MAM; however, the hypothesis of similar growth must also be tested in a co-culture to show resulting CFU numbers present the same result of no significant difference between the growth of wild-type and *⊿crtE*::kan *M. luteus*.

4.2 *Results*

4.2.1 WT versus *⊿crtE*::kan in Nutrient Rich (mNBE) Media

The competition growth curves for both wild-type and *⊿crtE*::kan *M. luteus* co-cultured in liquid mNBE media were obtained by removing, diluting, and plating culture samples every twelve hours for a total of at least three days and then measuring the CFU numbers. The CFUs were produced by plating 100 µL of

diluted sample on an mNBE agar plate; this was repeated three times (three technical replicates) for the four biological replicates every twelve hours. The raw data recorded for each technical replicate of the four mNBE media co-cultured biological replicates (OD_{600}, *ΔcrtE* (white) CFU, wild-type (yellow) CFU, total CFU, *ΔcrtE* and wild-type CFU ratios) is presented in the appendix as Tables A25-A28. To analyze the data the Log_{10} (CFU/mL) for each technical replicate (presented in appendix Tables A29-A32) was calculated using the equation:

Log_{10} ((CFU number X 10^ dilution) / (volume plated in mL))

For *ΔcrtE*::kan and WT *M. luteus*, the overall average and standard deviation of the Log_{10} (CFU/mL) for the three technical replicates of all four biological replicates (n = 12) was calculated and is presented numerically in Table 4.1 and as growth curves in Figure 4.1. The average and standard deviation of the WT and *ΔcrtE* CFU ratios (Table 4.2) was calculated using the WT and *ΔcrtE* CFU ratios of the three technical replicates in all four biological replicates (n = 12) and represented graphically in Figure 4.2. The fitness of *ΔcrtE*::kan relative to wild-type *M. luteus* was calculated using the fitness equation:

$$W = \frac{ln\left(\frac{A_f}{A_i}\right)}{ln\left(\frac{B_f}{B_i}\right)}$$

Where *W* is the fitness, *A* is the population size for the white *ΔcrtE*::kan strain, measured in CFU/mL. *B* is the population size for the yellow wild-type strain, also

measured in CFU/mL. The subscripts *i* and *f* are the initial and final sampling time points for the competition experiments.

The relative fitness for all replicates (n = 12) was determined to be 1.026 ± 0.049.

Table 4.1: Average and Standard Deviation Log_{10} (CFU/mL) Values for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in mNBE Media

Time (Days)	Avg Log_{10} (CFU/mL) of WT Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) WT Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.
0	5.784304087	5.79833441	0.18860585	0.199835152
0.5	7.556398117	7.502829026	0.196454299	0.225172146
1	9.451228026	9.486842285	0.399279782	0.39234969
1.5	9.366353388	9.347637182	0.198294333	0.249359153
2	8.418646642	8.45835009	0.288487385	0.241012349
2.5	9.66984833	9.705289433	0.681769767	0.589055756
3	8.58337191	8.67051796	0.214541902	0.206520833

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the four biological replicates at each sampling time for the wild-type versus Δ*crt*E::kan batch cultures in liquid mNBE media. This data was calculated from the raw data in Tables A25-A28 of the appendix.

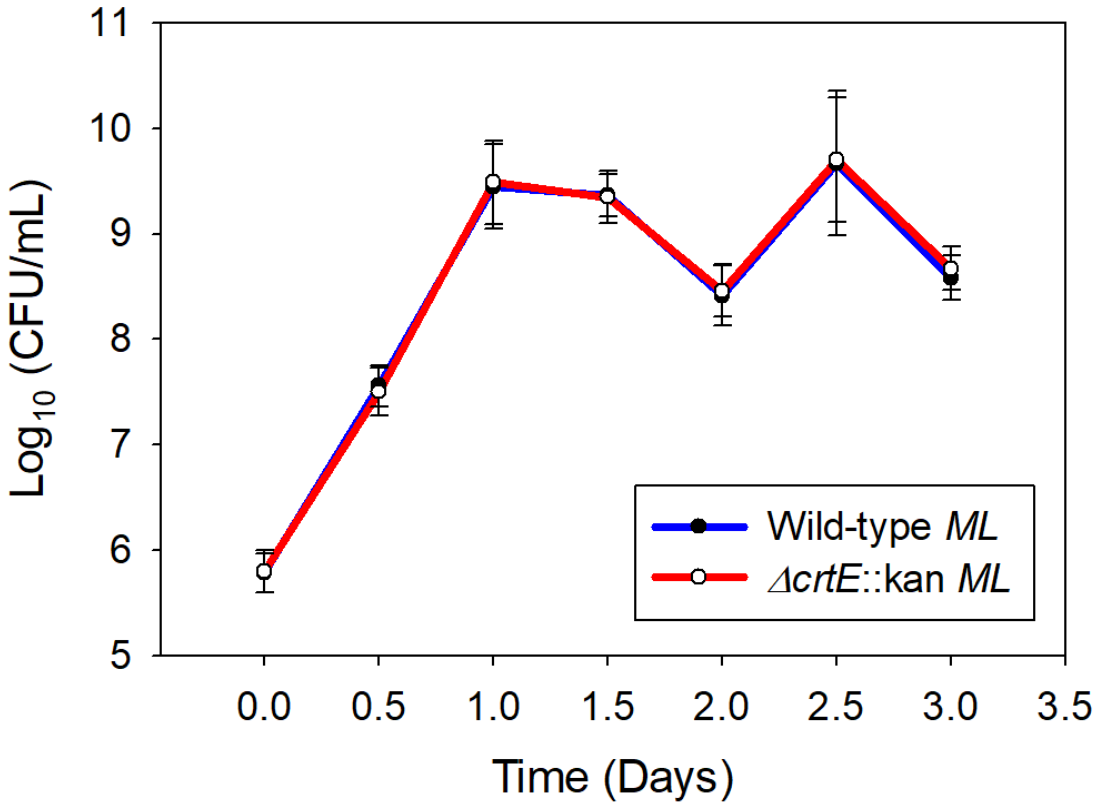

Figure 4.1: **Competition Growth Curves with Wild-type and *ΔcrtE::*kan *M. luteus* Co-cultured in Liquid mNBE Media.**

This figure graphically depicts the information presented in Table 4.1. Both the wild-type and *ΔcrtE*::kan *M. luteus* strains show identical growth patterns while co-cultured in nutrient rich (mNBE) media.

Table 4.2: Average and Standard Deviation CFU Ratios for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in mNBE Media

Time (Days)	Avg CFU Ratios from WT Biol. Reps.	Avg CFU Ratios from *ΔcrtE* Biol. Reps.	STDEV of CFU Ratios from WT Biol. Reps.	STDEV of CFU Ratios from *ΔcrtE* Biol. Reps.
0	0.49232861	0.50767139	0.062318717	0.062318717
0.5	0.530220814	0.469779186	0.066956569	0.066956569
1	0.479747057	0.520252943	0.069452629	0.069452629
1.5	0.510647518	0.489352482	0.084885901	0.084885901
2	0.478521592	0.521478408	0.083812473	0.083812473
2.5	0.480263958	0.519736042	0.069185189	0.069185189
3	0.450376937	0.549623063	0.051108882	0.051108882

This table shows the average and standard deviation of the WT and *ΔcrtE* CFU ratios of the three technical replicates for all four biological replicates (n = 12) while co-cultured in liquid mNBE media. These averages and standard deviations were calculated from the raw data presented in Tables A25-A28 of the appendix.

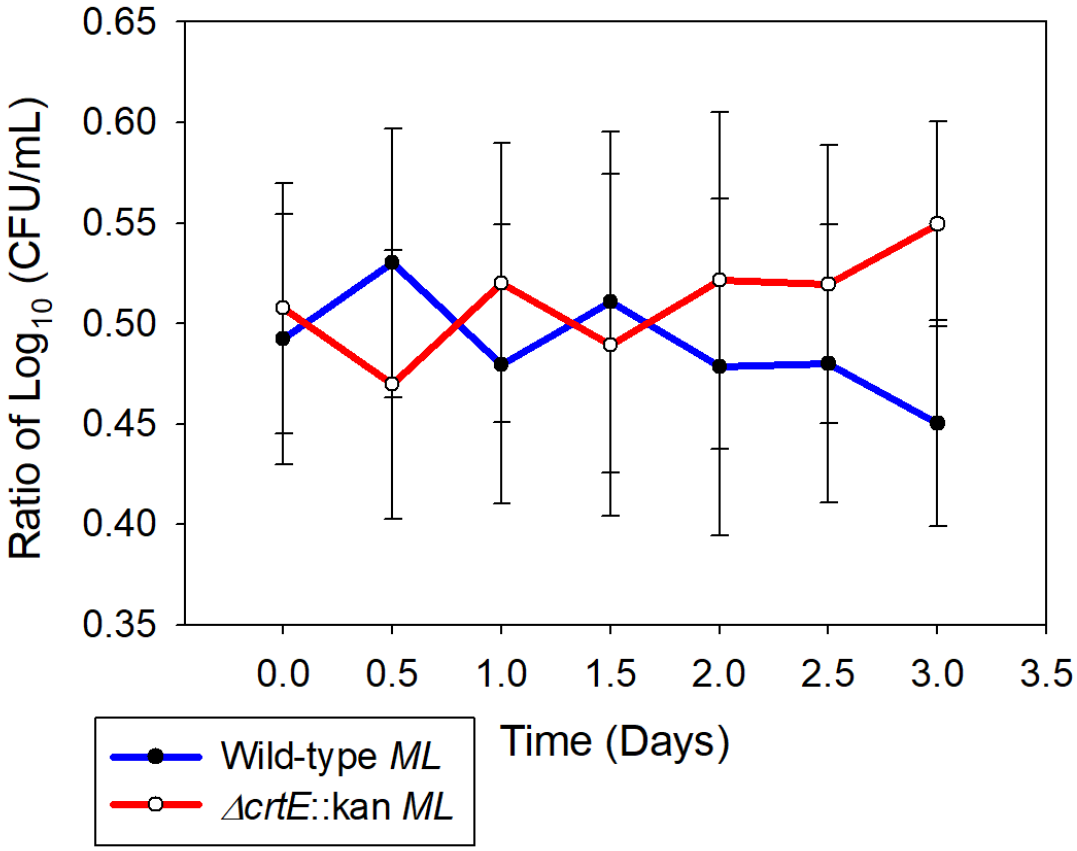

Figure 4.2: CFU Ratios for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in mNBE Media.

This figure depicts the information in Table 4.2. The CFU ratios of wild-type and *ΔcrtE*::kan *M. luteus* fluctuate slightly, however remain nearly identical.

4.2.2 WT versus *ΔcrtE*::kan in Nutrient Poor (MAM) Media

The competition growth curves for both wild-type and *ΔcrtE*::kan *M. luteus* co-cultured in liquid MAM media were obtained by removing, diluting, and plating culture samples every 24-48 h (24 h for the first three days and 48 h from days 5 to 7), then measuring the CFU. The CFUs were produced by plating 100 µL of diluted sample on an agar plate; this was repeated three times (three technical replicates) for the four biological replicates. The data recorded for each technical replicate of the four minimal acetate media co-cultured biological replicates (OD_{600}, *ΔcrtE* (white) CFU number, wild-type (yellow) CFU number, total CFU number, *ΔcrtE* and wild-type CFU ratios) is presented in the appendix as Tables A33-A36. To analyze the data the Log_{10} (CFU/mL) for each technical replicate (presented in Tables A37-A40 of the appendix) was calculated using the same equation in section 4.2.1:

Log_{10} ((CFU number X 10^ dilution) / (volume plated in mL))

For *ΔcrtE*::kan and WT *M. luteus*, the overall average and standard deviation of the Log_{10} (CFU/mL) for the three technical replicates of all four biological replicates (n = 12) was calculated and is presented numerically in Table 4.3 and graphically in Figure 4.3.

The average and standard deviation of the WT and *ΔcrtE* CFU ratios (Table 4.4) was calculated using the WT and *ΔcrtE* CFU ratios of the three technical replicates in all four biological replicates (n = 12) and represented graphically in Figure 4.4.

As in the mNBE competition experiment, the relative fitness between wild-type and *⊿crtE*::kan *M. luteus* strains was calculated using the fitness equation:

$$W = \frac{ln\left(\frac{A_f}{A_i}\right)}{ln\left(\frac{B_f}{B_i}\right)}$$

For competition experiments in nutrient deficient media (MAM), the relative fitness of *⊿crtE*::kan *M. luteus* for all replicates (n = 12) was calculated as 1.21 ± 0.18.

Table 4.3: Average and Standard Deviation Log_{10} (CFU/mL) Values for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in MAM Media

Time (Days)	Avg Log_{10} (CFU/mL) of WT Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) WT Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.
0	5.756407363	5.80548199	0.13259476	0.09190545
1	7.937224373	8.123807984	0.263706376	0.204690944
2	8.434000896	8.573286235	0.211640161	0.21589751
3	8.237091935	8.48257238	0.259018909	0.201227133
5	8.239705003	8.434269961	0.30071096	0.256177777
7	7.364603607	7.708050519	0.291495054	0.248960753

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the four biological replicates at each sampling time for the wild-type versus Δ*crt*E::kan batch cultures in liquid MAM media. This data was calculated from the raw data in Tables A33-A36 of the appendix.

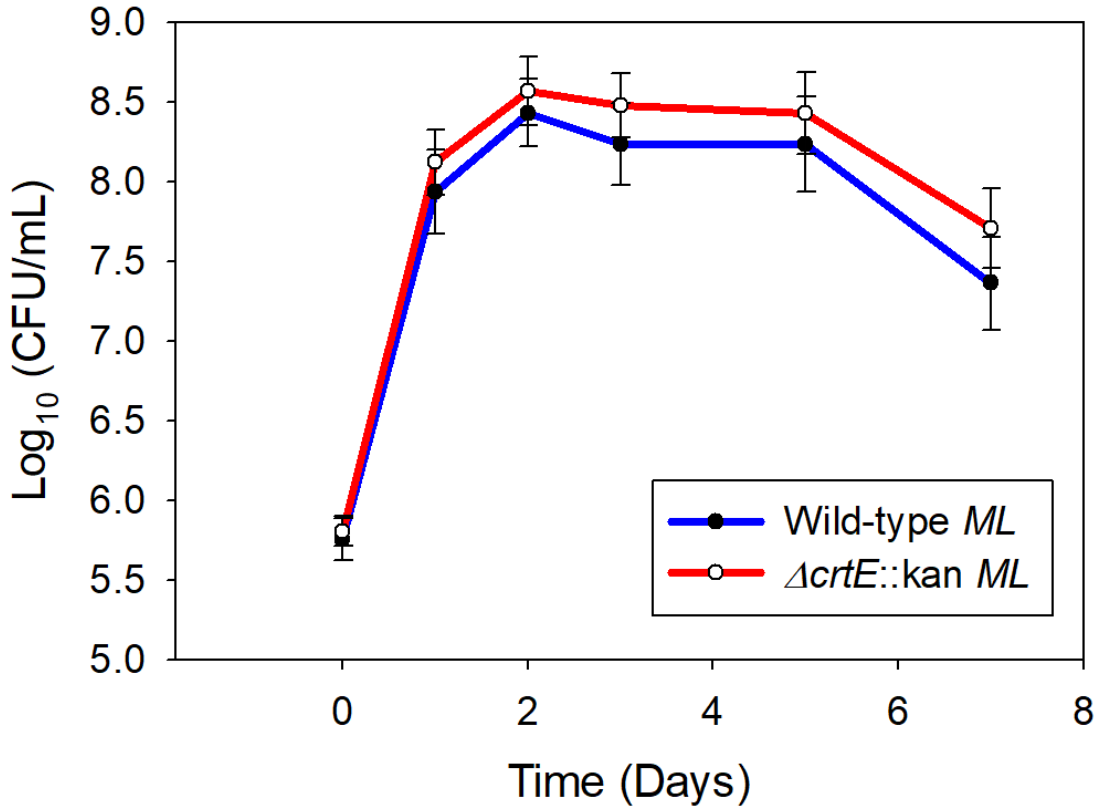

Figure 4.3: Competition Growth Curves with Wild-type and *ΔcrtE::*kan *M. luteus* Co-cultured in Liquid MAM Media.

This figure graphically depicts the information presented in Table 4.3. Both the wild-type and *ΔcrtE*::kan *M. luteus* strains show similar growth patterns while co-cultured in nutrient poor (MAM) media. The *ΔcrtE*::kan strain produced higher cell numbers over the seven-day experiment.

Table 4.4: Average and Standard Deviation CFU Ratios for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in MAM Media

Time (Days)	Avg CFU Ratios from WT Biol. Reps.	Avg CFU Ratios from *ΔcrtE* Biol. Reps.	STDEV of CFU Ratios from WT Biol. Reps.	STDEV of CFU Ratios from *ΔcrtE* Biol. Reps.
0	0.472368859	0.527631141	0.061558236	0.061558236
1	0.394986211	0.605013789	0.045031214	0.045031214
2	0.422661689	0.577338311	0.082157221	0.082157221
3	0.366435342	0.633564658	0.090782044	0.090782044
5	0.390173972	0.609826028	0.028753827	0.028753827
7	0.317043149	0.682956851	0.074397902	0.074397902

This table shows the average and standard deviation of the WT and *ΔcrtE* CFU ratios of the three technical replicates for all four biological replicates (n = 12) while co-cultured in liquid MAM media. These averages and standard deviations were calculated from the raw data presented in Tables A33-A36 of the appendix.

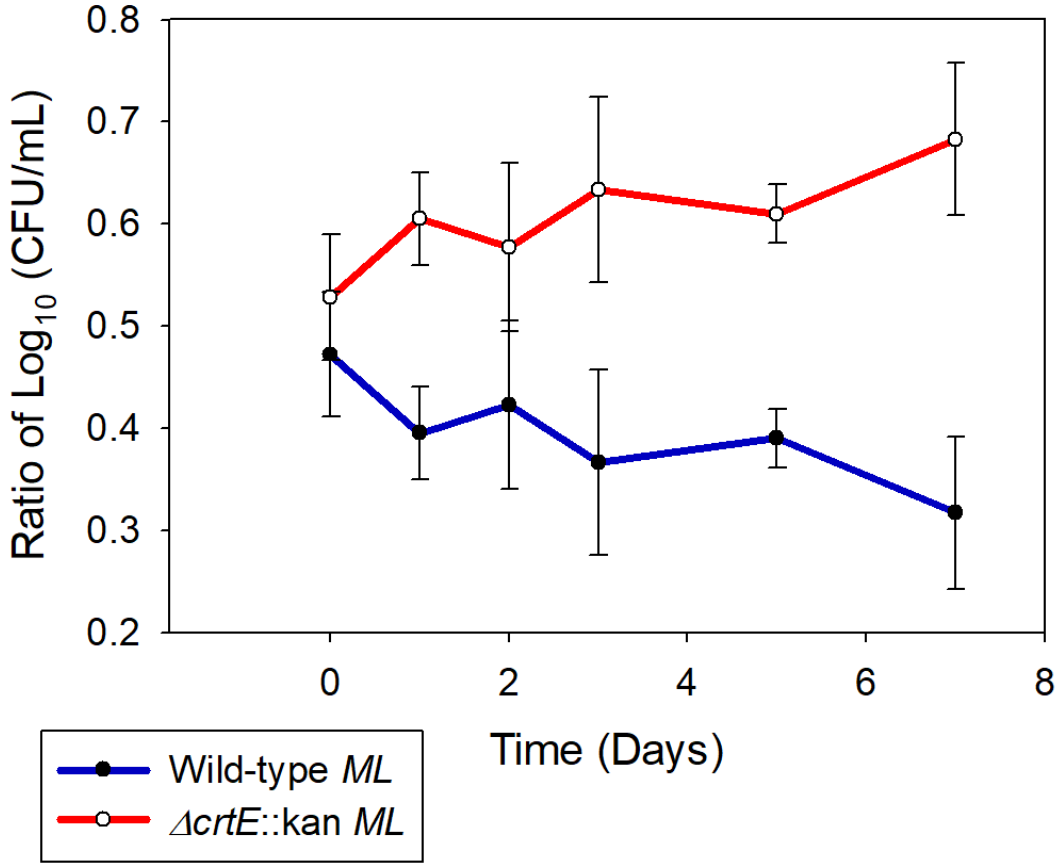

Figure 4.4: CFU Ratios for Wild-type and *ΔcrtE*::kan *M. luteus* During Co-cultured Growth in MAM Media.

This figure depicts the information in Table 4.4. The CFU ratios of wild-type and *ΔcrtE*::kan *M. luteus* initially start off with *ΔcrtE*::kan out competing the wild-type strain, this increases slightly overtime.

4.2.3 *ΔcrtE*::kan versus *ΔuspA616*::kan in Nutrient Rich (mNBE) Media

The competition growth curves for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* co-cultured in mNBE media were obtained by removing, diluting, and plating culture samples every 12 h for a total of at least three days and then measuring the CFU numbers. The CFUs were produced by plating 100 µL of diluted sample on an agar plate; this was repeated three times (three technical replicates) for the three biological replicates every twelve hours. The data recorded for each technical replicate of the three co-cultures (OD_{600}, *ΔcrtE* (white) CFU number, *ΔuspA616* (yellow) CFU number, total CFU number, *ΔcrtE* and *ΔuspA616* CFU ratios) is presented in the appendix as Tables A41-A43. To analyze the data the Log_{10} (CFU/mL) for each technical replicate (presented in Tables A44-A46 of the appendix) was calculated using the same equation in sections 4.2.1 and 4.2.2:

Log_{10} ((CFU number X 10^ dilution) / (volume plated in mL))

For *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus*, the overall average and standard deviation of the Log_{10} (CFU/mL) for the three technical replicates of all three biological replicates (n = 9) was calculated and is presented numerically in Table 4.5 and as growth curves in Figure 4.5.

The average and standard deviation of the *ΔcrtE* and *ΔuspA616* CFU ratios (Table 4.6) was calculated using the *ΔcrtE* and *ΔuspA616* CFU ratios of the three technical replicates in all three biological replicates (n = 9) and represented graphically in Figure 4.6.

Using the fitness equation:

$$W = \frac{ln\left(\frac{A_f}{A_i}\right)}{ln\left(\frac{B_f}{B_i}\right)}$$

Where *W* is the fitness, *A* is the population size for the yellow *ΔuspA616*::kan strain, measured in CFU/mL. *B* is the population size for the white *ΔcrtE*::kan strain, also measured in CFU/mL. The subscripts *i* and *f* are the initial and final sampling time points for the competition experiments.

The relative fitness for all replicates (n = 9) was determined to be 0.97 ± 0.043 for *ΔuspA616*::kan *M. luteus* relative to the *ΔcrtE*::kan strain in liquid mNBE media.

Table 4.5: Average and Standard Deviation Log_{10} (CFU/mL) Values for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in mNBE Media

Time (Days)	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔuspA616* Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔuspA616* Biol. Reps.
0	6.185724652	6.061885107	0.0617583	0.0969795
0.5	6.709277813	6.563963323	0.129047917	0.13680933
1	7.95545616	7.793681131	0.112549077	0.128392919
1.5	8.607198424	8.441367716	0.136041772	0.18001208
2	8.536688754	8.338191618	0.21286159	0.197677602
2.5	9.043330928	8.933663929	0.162449721	0.157529255
3	8.464239046	8.261580168	0.142351951	0.155391398

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the three biological replicates at each sampling time for the Δ*crt*E::kan versus *ΔuspA616*::kan batch cultures in liquid mNBE media. This data was calculated from the raw data in Tables A41-A43 of the appendix.

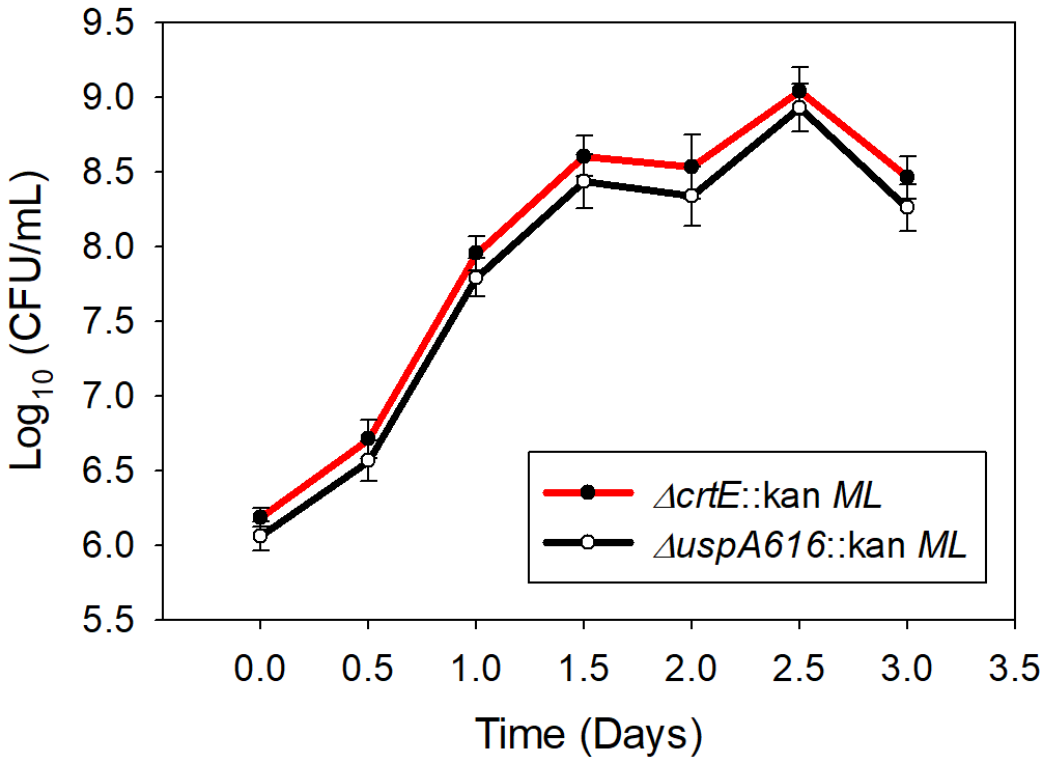

Figure 4.5: Competition Growth Curves with *ΔcrtE::*kan and *ΔuspA616*::kan *M. luteus* Co-cultured in Liquid mNBE Media.

This figure graphically depicts the information presented in Table 4.5. Both the *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* strains show similar growth patterns while co-cultured in nutrient rich (mNBE) media.

Table 4.6: Average and Standard Deviation CFU Ratios for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in mNBE Media

Time (Days)	Avg CFU Ratios from *ΔcrtE* Biol. Reps.	Avg CFU Ratios from *ΔuspA616* Biol. Reps.	STDEV of CFU Ratios from *ΔcrtE* Biol. Reps.	STDEV of CFU Ratios from *ΔuspA616* Biol. Reps.
0	0.570458083	0.429541917	0.03689505	0.036895046
0.5	0.582173432	0.417826568	0.0466795	0.046679497
1	0.591702143	0.408297857	0.03214641	0.032146408
1.5	0.592755053	0.407244947	0.07431627	0.07431627
2	0.610072453	0.389927547	0.07104353	0.071043528
2.5	0.562474515	0.437525485	0.0402261	0.040226104
3	0.61336829	0.38663171	0.047854	0.047853996

This table shows the average and standard deviation of the *ΔcrtE* and *ΔuspA616* CFU ratios of the three technical replicates for all three biological replicates (n = 9) while co-cultured in liquid mNBE media. These averages and standard deviations were calculated from the raw data presented in Tables A41-A43 of the appendix.

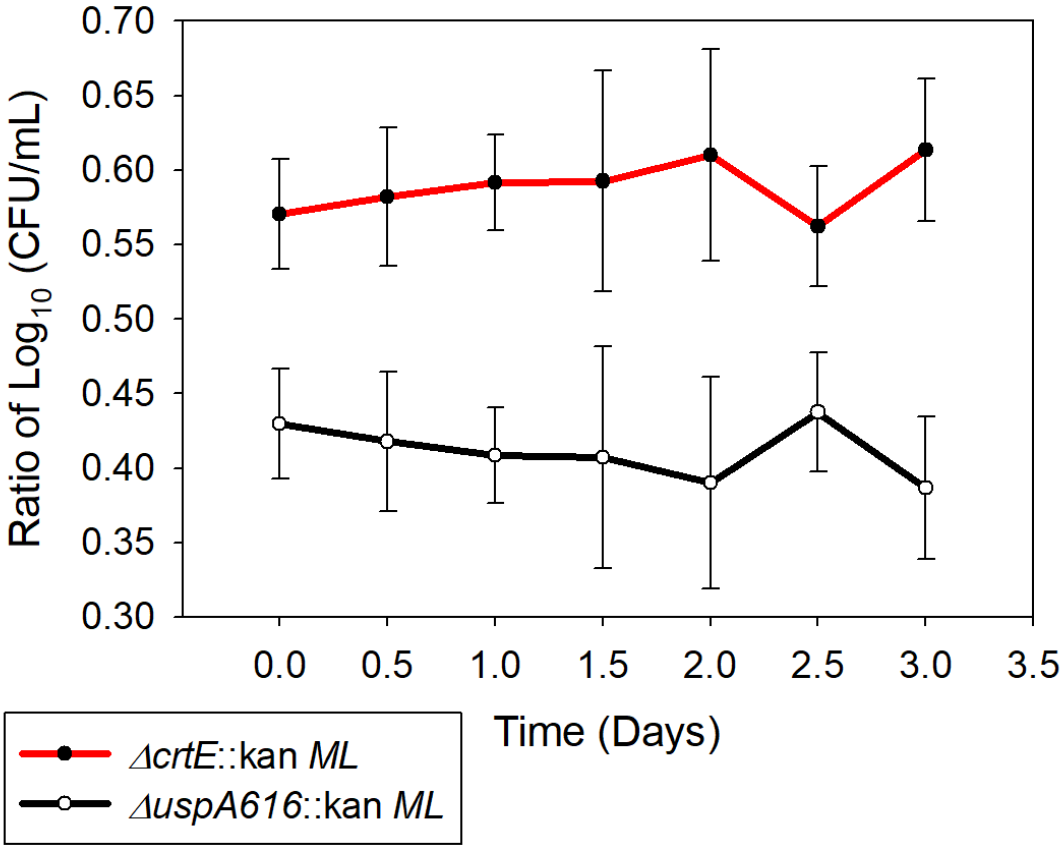

Figure 4.6: CFU Ratios for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in mNBE Media

This figure depicts the information in Table 4.6. The CFU ratios of *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* remain relatively constant, the non-equal ratios suggest an unequal number of cells add to the co-cultures from the starter cultures.

4.2.4 *ΔcrtE*::kan versus *ΔuspA616*::kan in Nutrient Poor (MAM) Media

The competition growth curves for *ΔcrtE::*kan and *ΔuspA616*::kan *M. luteus* co-cultured in minimal acetate media were obtained by removing, diluting, and plating culture samples every 48 hours over a course of 14 days. The CFU data was produced by dot plating (the same method used for individual growth curves in Chapter 3) of 20 µL of diluted sample on an mNBE agar plate. Each of the three co-cultured biological replicates had six 20 µL dots (six technical replicates) at a specific dilution. The data recorded for each technical replicate (time of sample, dilution, *ΔuspA616* [yellow] CFU number, *ΔcrtE* [white] CFU number) as well as the average *ΔuspA616* [yellow] CFU number, average *ΔcrtE* [white] CFU number, and average sum of the yellow and white CFUs are presented in the appendix as Tables A47-A49.

To analyze the data the Log_{10} (average yellow CFU/mL) and Log_{10} (average white CFU/mL) for each biological replicate (presented in Table A50 of the appendix) was calculated using the same equation in sections 4.2.1, 4.2.2, and 4.2.3:

Log_{10} ((CFU number X 10^ dilution) / (volume plated in mL))

The overall average and standard deviation Log_{10} (CFU/mL) of the three biological replicates was then calculated and is presented numerically in Table 4.7 and as growth curves in Figure 4.7. The average and standard deviation of the *ΔuspA616* and *ΔcrtE CFU* ratios (Table 4.8) was calculated using the

ΔuspA616 and *ΔcrtE* CFU ratios of the six technical replicates in the three biological replicates (n = 18) and represented graphically in Figure 4.8.

As in the mNBE competition experiment, the relative fitness between the *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* strains was calculated using the fitness equation:

$$W = \frac{ln\left(\frac{A_f}{A_i}\right)}{ln\left(\frac{B_f}{B_i}\right)}$$

For competition experiments in nutrient deficient media (MAM), the relative fitness ratio of *ΔuspA616*::kan for all replicates (n = 18) was -0.164 ± 0.231.

Table 4.7: Average and Standard Deviation Log_{10} (CFU/mL) Values for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in MAM Media

Time (Days)	Avg Log_{10} (CFU/mL) of *ΔcrtE* Biol. Reps.	Avg Log_{10} (CFU/mL) of *ΔuspA616* Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔcrtE* Biol. Reps.	STDEV of Log_{10} (CFU/mL) *ΔuspA616* Biol. Reps.
0	5.791	5.858	0.019	0.020
2	7.364	7.073	0.323	0.217
4	8.134	7.788	0.348	0.263
6	1.943	7.519	1.989	0.183
8	1.943	7.175	1.989	0.254
10	1.276	6.142	1.322	0.617
12	1.435	6.219	1.481	0.519
14	1.276	6.327	1.322	0.503

This table displays the average Log_{10} (CFU/mL) and standard deviation of the Log_{10} (CFU/mL) values from the three biological replicates at each sampling time for the Δ*crt*E::kan versus *ΔuspA616*::kan batch cultures in liquid MAM media. This data was calculated from the raw data in Tables A47-A49 of the appendix.

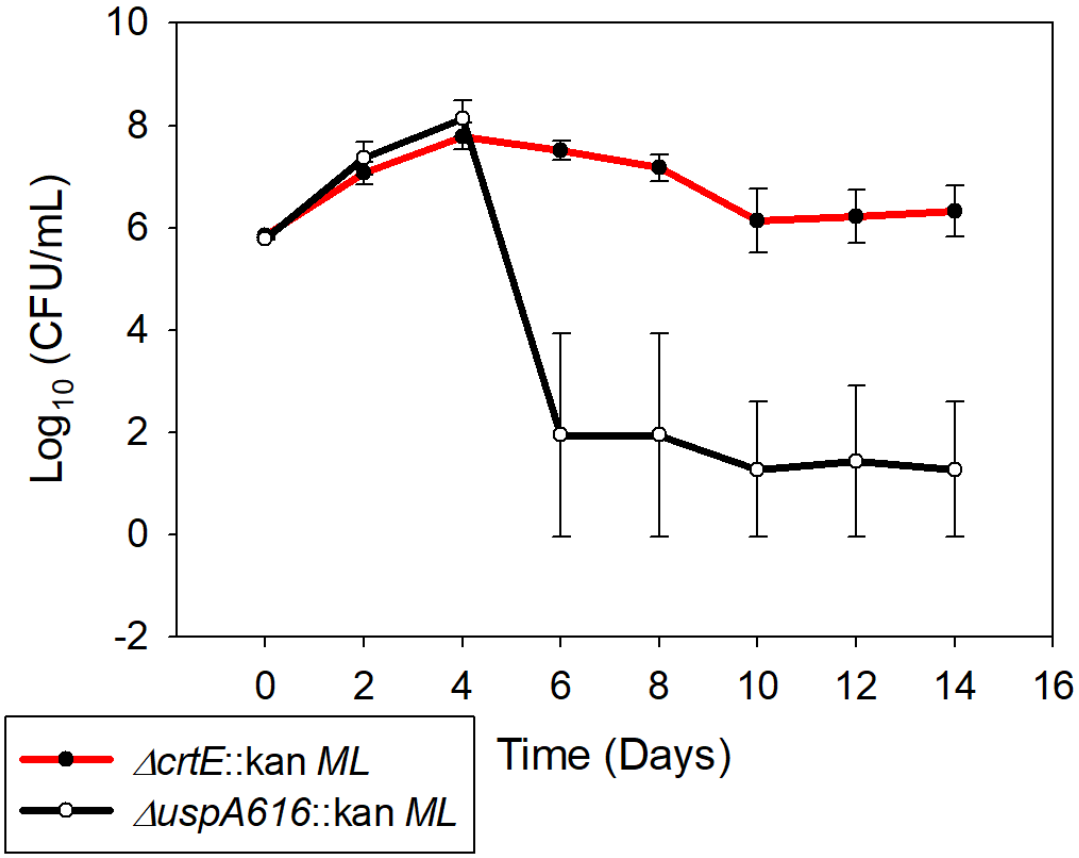

Figure 4.7: Competition Growth Curves with *ΔcrtE::*kan and *ΔuspA616*::kan *M. luteus* Co-cultured in Liquid mNBE Media.

This figure graphically depicts the information presented in Table 4.7. While the *ΔuspA616*::kan strain slightly outcompetes *ΔcrtE*::kan *M. luteus*, after day four, there is a significant drop in culturability, and therefore CFU number from the *ΔuspA616*::kan strain compared to *ΔcrtE*::kan *M. luteus* when co-cultured in liquid MAM media.

Table 4.8: Average and Standard Deviation CFU Ratios for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in MAM Media

Time (Days)	Avg CFU Ratios from *ΔcrtE* Biol. Reps.	Avg CFU Ratios from *ΔuspA616* Biol. Reps.	STDEV of CFU Ratios from *ΔcrtE* Biol. Reps.	STDEV of CFU Ratios from *ΔuspA616* Biol. Reps.
0	0.462	0.538	0.004	0.004
2	0.656	0.344	0.056	0.056
4	0.658	0.342	0.163	0.163
6	0.008	0.992	0.008	0.008
8	0.006	0.994	0.006	0.006
10	0.020	0.980	0.020	0.020
12	0.030	0.970	0.030	0.030
14	0.001	0.999	0.001	0.001

This table shows the average and standard deviation of the *ΔcrtE* and *ΔuspA616* CFU ratios of the six technical replicates in the three biological replicates (n = 18) at each sampling time.

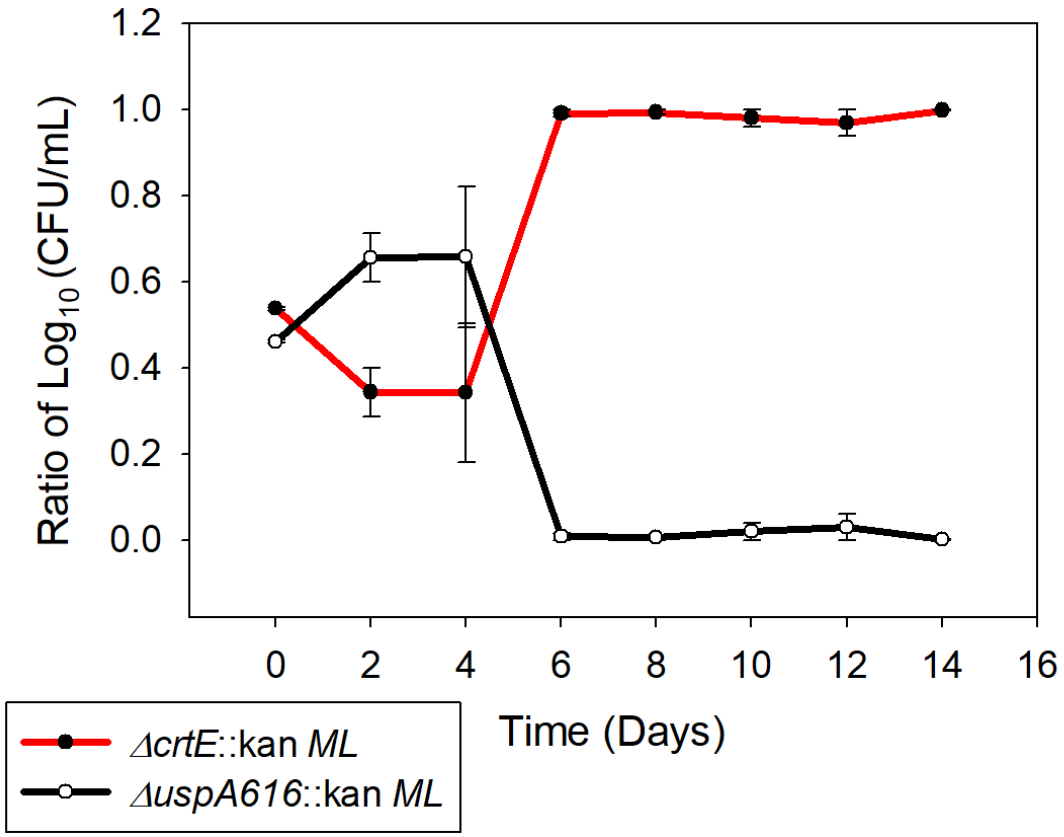

Figure 4.8: CFU Ratios for *ΔcrtE*::kan and *ΔuspA616*::kan *M. luteus* During Co-cultured Growth in MAM Media

This figure depicts the information in Table 4.8. The CFU ratios show *ΔuspA616*::kan initially outcompetes *ΔcrtE*::kan in the MAM co-culture, however after day four, the CFU numbers for *ΔuspA616*::kan drastically decrease compared to *ΔcrtE*::kan suggesting loss of culturability under nutrient deprived environments such as MAM media.

4.3 *Discussion and Conclusion*

It was hypothesized the *crtE* gene would have no influence on exponential growth *of M. luteus* under optimum growth conditions (in mNBE media), nor influence the dormant stress response caused by nutrient starvation (in MAM media). To test this hypothesis, the growth of *ΔcrtE*::kan and wild-type *M. luteus* were measured in a carbon abundant media (liquid mNBE) as well as a medium that has been shown to consistently induce latency in *M. luteus* cells, minimal acetate media (MAM). Like the individual growth curve data, the wild-type versus *ΔcrtE*::kan *M. luteus* competition results show the growth of *ΔcrtE*::kan *M. luteus* is identical to wild-type in nutrient rich media (mNBE) (Figure 4.1). This is also supported by the calculated fitness ratio which has a value of 1.026 ± 0.049.

However, the results of the competition experiment in MAM showed a slight advantage in fitness for *ΔcrtE*::kan over wild-type as seen in Figure 4.3 and by the fitness ratio of 1.21 ± 0.18, whereas in the MAM isolated growth curve data showed a slight advantage for wild-type over *ΔcrtE*::kan (Figure 3.2). It is unclear if this difference in trend is due to slight changes in the initial inoculation of the two strains, or if there are small changes in the growth of *ΔcrtE*::kan or wild-type *M. luteus* depending on if they are grown in isolated environments, or in co-culture together. Compared to the nutrient rich media, MAM produced significant decreases in the CFU numbers over time, indicating both wild-type and *ΔcrtE*::kan strains transitioned into latency as a response to scarcity of a carbon source. These experiments suggest the *crtE* gene is a relatively neutral site that

may be used as a knockin site for future gene rescue experiments. This also allows the use of the *⊿crtE::*kan mutant as a wild-type substitute in competition experiments against other *M. luteus* mutants to evaluate the fitness of such mutants. Of particular interest is the competition experiments against *M. luteus* mutants of proteins that have been shown to have significantly different concentrations whether the cells are exponential or dormant phases.

One such protein, UspA616, has been shown (by mass spectroscopy proteomics), to be significantly upregulated in dormant cells as opposed to exponentially growing cells. Because of the increased concentration of UspA616 in dormant cells, it was hypothesized that the UspA616 protein plays an important role in the dormancy mechanisms, and therefore the ablation of this gene would negatively impact the ability of *M. luteus* to adapt its metabolism for surviving nutrient deprivation. Because *⊿uspA616*::kan is yellow, the competition of *⊿crtE*::kan (white) versus *⊿uspA616*::kan would allow easy differentiation between the two colony forming units. The competition experiment in both nutrient rich and nutrient poor media was used to test this hypothesis. Figure 4.5 shows both bacterial growth curves look almost identical in mNBE media. The competition experiment was repeated for minimal acetate media, with the only difference being the process of gathering cell number information. Results from the mNBE competition show *⊿crtE*::kan had slightly higher CFU numbers than *⊿uspA616*::kan, however the growth trends for both strains follow similar patterns (Figure 4.5) and neither was outcompeted which can be seen more clearly in the

ratio graph (Figure 4.6), as the ratio of CFU number remain relatively constant over three days. The fitness ratio also shows the fitness similarities with a value of 0.97 ± 0.043. The higher *ΔcrtE*::kan CFU number may be from adding more *ΔcrtE*::kan cells during the initial inoculation of the co-cultures, which is also suggested by Figures 4.5 and 4.6 as the zero hour CFU counts were slightly different between the two *M. luteus* strains. Overall growth of both *ΔcrtE*::kan and *ΔuspA616*::kan were similar in nutrient rich media, suggesting the knockout of *ΔuspA616*::kan does not have a negative impact on growth of *M. luteus* in optimum growth conditions, and therefore the UspA616 protein does not have a significant effect on cellular processes of exponentially growing cells.

Preliminary experiments showed a longer lag time for the *ΔuspA616* mutant compared to the wild-type and *ΔcrtE* mutant, therefore we predicted this experiment would extend longer than other MAM competition assays. Because this experiment was expected to grow for longer times to reach dormancy, we used dot plating to produce CFU data. Instead of plating 100 µL of diluted sample on the entire plate (one technical replicate per plate), multiple 20 µL dots were placed on each plate (multiple technical replicates per plate). This process allowed the completion of this experiment with less materials. The results from the nutrient starvation competition conditions show that *ΔuspA616*::kan *M. luteus* experienced similar growth to *ΔcrtE*::kan *M. luteus* during initial log growth phases (days 0-4). In fact, *ΔuspA616*::kan was originally outcompeting *ΔcrtE*::kan during this time as seen in both Figure 4.7 and 4.8; however, after day 4 the

ΔuspA616::kan experienced a severe decrease in CFU numbers. The point at which the *ΔuspA616*::kan mutant loses culturability occurs right as the *ΔcrtE*::kan strain first experiences a decrease in growth (Figure 4.7), as it transitioned into the stationary phase. The fitness ratio is -0.164 ± 0.231, which is significantly less than 1. This suggests the *uspA616* gene is critical for cell division as cells transition into a stationary phase as well as a dormant phase.

This loss of culturability could be explained by two mechanism, 1) lack of the *uspA616* gene may cause cells to enter a dormant state (and therefore cease cell replication) or 2) loss of the *uspA616* gene causes death in cells experiencing nutrient stresses. Preliminary viability assays suggest the dormant *ΔuspA616*::kan cells die; however more studies are needed to determine the exact mechanism causing decreased cell division of *ΔuspA616*::kan cells during starvation stress and definitively prove death as the cause for loss of capturability in the *uspA616* mutant.

Conclusion

Like many stress response systems, dormancy is a complex process involving multiple levels of gene and protein engagements. Because some pathogenic bacteria can increase their antibiotic tolerance, understanding the mechanism of dormancy is medically important. By studying dormancy in a simpler model organism, *M. luteus*, it is hoped that a general mechanism of dormancy could be elucidated that is applicable to dormancy mechanisms in pathogenic bacteria.

The purpose of this project was to develop a simple colorimetric competition assay as a means for studying phenotypic differences between suspected dormant gene knockouts and the "wild-type substitute" *ΔcrtE*::kan *M. luteus*. First, individual and co-culture growth curve data, were able to demonstrate identical growth ability between *ΔcrtE*::kan and wild-type *M. luteus* in nutrient rich media. Individual and co-cultured growth of *M. luteus* in nutrient minimal media showed only slight differences in growth between the two strains. Notably the *ΔcrtE*::kan strain showed similar growth patterns under conditions that induce dormancy in wild-type *M. luteus*, displaying its ability to be used in dormancy competition experiments. The colorimetric competition assay was then put to its first test by evaluating the relative fitness of the *uspA616* gene knockout mutant compared to the *crtE* mutant. There were no significant growth differences between the two strains in nutrient rich media, suggesting the UspA616 protein does not have a significant effect on cellular processes of exponentially growing cells. However, there was a significant fitness decrease in *ΔuspA616*::kan cells in MAM showing

there is functional importance to the UspA616 protein when cells encounter nutrient deficient environments.

Two other proteins that were found to be upregulated in dormant *M. luteus* cells were isocitrate lyase and malate synthase (Mali et al., 2017). Both of these proteins are key enzymes involved in the glyoxylate cycle (Dolan and Welch, 2018). The glyoxylate cycle, also called the glyoxylate shunt, allows microorganisms to use simple carbon compounds, such as acetate, as a carbon source when glucose is not available. Acetate is used to cause *M. luteus* to enter dormancy; however, this also likely causes *M. luteus* to switch metabolism from the citric acid cycle to the glyoxylate cycle as a main pathway for anabolism (Figure 4.9). One proposal for why the *uspA616* gene knockout would cause death to cells in minimal acetate media, is because the UspA616 protein is involved in the activation of the glyoxylate pathway, possibly as a transcription factor, or by promoting the activation of transcriptions factors needed to synthesize proteins involved in the glyoxylate shunt (like isocitrate lyase or malate synthase). To test if the UspA616 protein directly or indirectly regulates other proteins needed for the glyoxylate pathway, future experiments could use proteomic analysis to compare the protein concentrations of dormant *ΔuspA616*::kan cells to the proteomic data already acquired form dormant wild-type cells.

Of further interest, one of the other Usp proteins, UspA712, is upregulated in exponentially growing cells, while not expressed in dormant cells. Preliminary

data on the UspA712 knockout mutant (*ΔuspA712*::kan), gathered by graduate students Abi Bodunrin and Spencer Havis, suggests it grows much better in liquid acetate minimal media than in media with a complex carbon source including liquid mNBE and glucose. This suggests that the UspA712 protein may be complementary and opposing to UspA616 as a regulatory factor for changing carbon flow into the glyoxylate cycle.

Competition experiments for the *ΔuspA712*::kan and *ΔuspA184*::kan mutants are planned for the near future. The other 17 proteins previously implicated in the dormant phenotype are candidates for future competition assays once knockout mutants are generated. These competition assays will allow the characterization of different mutant phenotypes and will therefore help elucidate a general mechanism controlling stress responses in bacteria that utilize dormancy as a survival mechanism.

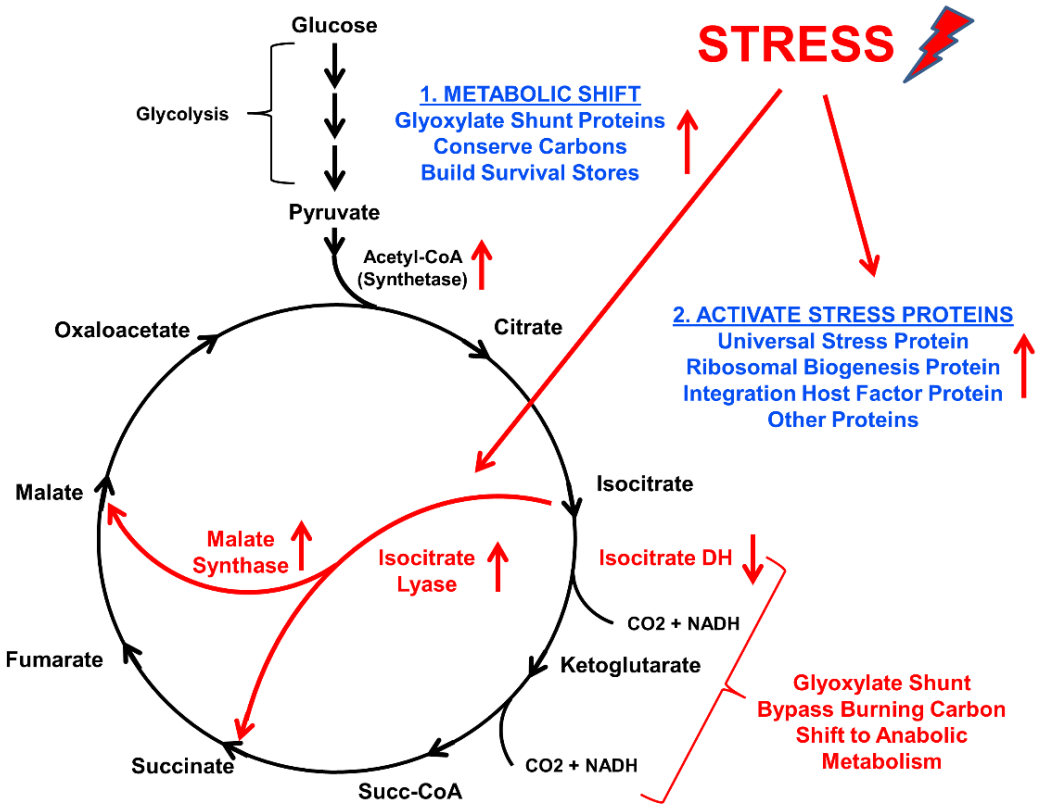

Figure 4.9: Overview of the Glyoxylate Shunt in *Micrococcus luteus*

This is a diagram depicts the upregulation of the glyoxylate pathway. The citric acid cycle (TCA) is shown in black. The glyoxylate pathway which bypasses the decarboxylation steps of the TCA is shown in red. Both pathways are identical until the conversion of isocitrate. In the citric acid cycle isocitrate is oxidized by isocitrate dehydrogenase (Isocitrate DH) to α-ketoglutarate, releasing one CO_2 molecule. However, in the glyoxylate pathway, isocitrate is cleaved by isocitrate lyase into succinate and glyoxylate (not shown). Malate synthase then catalyzes the condensation of glyoxylate and the acyl group of acetyl-CoA (not shown) producing malate. This figure also depicts the upregulation of isocitrate lyase and malate synthase and the downregulation of isocitrate DH in an effort to conserve carbon under nutrient stress.

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Appendix

Results of Wild-Type Isolated Growth Curve in Nutrient Rich (mNBE) Media

Table A1: Biological Replicate 1

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.03	4	4	2	5	5	5	9	3	3	3	4	7	4	4	4	8
0.5	1.47	6	10	7	9	12	16	9	10	11	13	16	12	13	12	14	13
1	6.54	6	16	13	12	16	15	11	15	9	13	14	15	11	11	12	14
1.5	8.19	6	9	13	16	14	10	9	15	9	9	11	15	8	12	9	10
2	5.94	6	23	25	17	14	18	17	18	16	9	13	10	18	18	18	18
2.5	7.06	6	28	29	37	27	27	28	26	38	39	30	33	25	23	26	36
3	5.71	6	10	8	14	10	17	11	6	6	12	3	6	13	5	9	12

Table A2: Biological Replicate 2

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.02	4	6	5	7	2	3	2	3	3	3	7	9	6	9	6	7
0.5	1.44	6	14	8	9	9	8	5	7	14	6	9	9	15	5	8	11
1	7.19	6	14	19	18	19	16	14	20	24	22	19	19	18	15	18	15
1.5	7.58	6	18	13	14	13	13	11	19	16	16	17	18	16	20	15	16
2	7.15	6	18	10	20	10	15	16	12	17	9	13	11	12	10	8	14
2.5	6.58	6	27	27	35	31	20	30	31	30	23	36	29	26	31	28	27
3	7.17	6	12	13	11	17	7	17	20	11	17	12	16	10	5	7	15

Table A3: Biological Replicate 3

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.04	4	4	6	2	3	3	2	2	4	4	3	4	6	4	4	3
0.5	1.34	6	3	4	9	3	6	4	9	8	9	3	14	13	8	7	6
1	6.99	6	16	18	15	25	18	17	27	18	21	20	23	15	15	23	17
1.5	6.93	6	13	13	10	13	10	12	15	16	14	14	16	8	24	11	12
2	6.66	6	17	20	17	17	9	15	20	10	14	13	23	15	16	19	18
2.5	5.94	6	12	9	13	13	11	12	15	17	15	13	15	16	12	11	12
3	6.20	6	21	26	20	19	20	16	17	18	21	16	24	17	21	19	14

Tables A1-A3: These tables show direct data gathered from each of the mNBE wild-type *M. luteus* isolated cultures every 12 hours over three days. Table A1, A2, and A3 are the biological replicates 1, 2, and 3 respectively. The optical density, serial dilution, and number of CFUs counted in each of the fifteen 10 µL dots for each 12-hour time sampling is reported. While all fifteen 10 µL dots are technical replicates, they were divided into three groups for easier data analysis where Group 1, Group 2, and Group 3 are colored blue, orange, and green respectively.

Table A4: Data Analysis of Biological Replicate 1

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	4.20	4.4	5.4	4.20E6	4.40E6	5.40E6	6.623	6.643	6.732	6.666
0.5	10.8	11.8	12.8	1.08E9	1.18E9	1.28E9	9.033	9.071	9.107	9.070
1	14.4	12.4	12.6	1.44E9	1.24E9	1.26E9	9.158	9.093	9.100	9.117
1.5	12.4	10.6	10.8	1.24E9	1.06E9	1.08E9	9.093	9.025	9.033	9.050
2	19.4	14.6	16.4	1.94E9	1.46E9	1.64E9	9.287	9.164	9.214	9.222
2.5	29.6	32.2	28.6	2.96E9	3.22E9	2.86E9	9.471	9.507	9.456	9.478
3	11.8	7.6	9.0	1.18E9	7.60E8	9.00E8	9.071	8.880	8.954	8.968

Table A5: Data Analysis of Biological Replicate 2

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	4.6	3.6	7.4	4.60E6	3.60E6	7.40E6	6.662	6.556	6.869	6.696
0.5	9.6	8.2	9.6	9.60E8	8.20E8	9.60E8	8.982	8.913	8.982	8.959
1	17.2	19.8	17	1.72E9	1.98E9	1.70E9	9.235	9.296	9.230	9.254
1.5	14.2	15.8	17	1.42E9	1.58E9	1.70E9	9.152	9.198	9.230	9.193
2	14.6	13.4	11	1.46E9	1.34E9	1.10E9	9.164	9.127	9.041	9.110
2.5	28	30	28.2	2.80E9	3.00E9	2.82E9	9.447	9.477	9.450	9.458
3	12	15.4	10.6	1.20E9	1.54E9	1.06E9	9.079	9.187	9.025	9.097

Table A6: Data Analysis of Biological Replicate 3

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	3.6	3	4.2	3.60E6	3.00E6	4.20E6	6.556	6.477	6.623	6.552
0.5	5	6.6	9.6	5.00E8	6.60E8	9.60E8	8.698	8.819	8.982	8.833
1	18.4	20.6	18.6	1.84E9	2.06E9	1.86E9	9.264	9.313	9.269	9.282
1.5	11.8	14.2	14.2	1.18E9	1.42E9	1.42E9	9.071	9.152	9.152	9.125
2	16	14.4	18.2	1.60E9	1.44E9	1.82E9	9.204	9.158	9.260	9.207
2.5	11.6	14.4	13.2	1.16E9	1.44E9	1.32E9	9.064	9.158	9.120	9.114
3	21.2	17.6	19	2.12E9	1.76E9	1.90E9	9.326	9.245	9.278	9.283

Tables A4-A6: Tables A4, A5, and A6, show further analysis of the data collected in Tables A1, A2, and A3 respectively. The average CFU from each group in Tables A1-A3 were calculated. This average CFU number was then back calculated to CFU/mL. The Log_{10} (CFU/mL) for each group was calculated, and an average Log_{10} (CFU/mL) from all group is given in the final column.

Results of *ΔcrtE*::kan Individual Growth Curve in Nutrient Rich (mNBE) Media

Table A7: Biological Replicate 1

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.03	4	2	1	4	3	5	6	4	2	3	7	2	2	3	3	7
0.5	1.48	6	18	13	13	15	17	11	17	11	11	15	14	14	12	16	15
1	7.97	6	13	15	12	13	16	14	12	8	15	17	19	15	15	13	9
1.5	6.74	6	9	13	16	14	10	9	15	9	9	11	15	8	12	9	6
2	7.81	6	19	13	17	21	17	23	20	17	15	15	21	15	19	18	24
2.5	5.73	6	20	22	25	14	27	31	20	21	27	13	21	23	20	19	23
3	7.26	6	35	42	25	25	19	34	26	17	24	18	25	19	17	25	19

Table A8: Biological Replicate 2

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.03	4	3	3	4	2	2	5	5	4	4	7	3	3	5	2	2
0.5	1.44	6	7	7	5	2	4	5	9	7	8	5	6	5	3	3	6
1	7.47	6	17	24	20	18	24	20	21	20	21	23	25	25	23	21	21
1.5	7.26	6	23	20	22	23	22	17	23	21	20	17	23	23	21	19	20
2	6.86	6	20	15	15	15	23	18	24	14	11	11	14	15	15	28	23
2.5	6.66	6	55	67	55	53	47	53	49	47	69	64	57	52	63	62	52
3	6.78	6	27	41	29	32	35	40	38	21	26	35	41	28	37	27	36

Table A9: Biological Replicate 3

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.03	4	2	1	2	2	3	1	6	5	3	4	2	2	2	4	5
0.5	1.58	6	4	4	4	4	5	8	4	4	9	8	4	5	7	4	4
1	6.90	6	22	25	29	26	33	22	22	23	30	27	30	22	25	32	33
1.5	7.13	6	15	18	11	14	13	11	13	12	17	15	14	14	13	14	15
2	6.68	6	15	15	14	22	21	19	13	21	15	14	16	13	18	21	14
2.5	6.01	6	23	27	34	26	24	31	32	30	33	27	27	28	26	34	30
3	6.20	6	33	49	26	25	18	40	44	20	27	30	17	28	25	30	17

Tables A7-A9: These tables show direct data gathered from each of the three rich media Δ*crt*E::kan *M. luteus* biological replicates every 12 hours over the course of three days. Table A7, A8, and A9 are the biological replicates 1, 2, and 3 respectively. The optical density, serial dilution, and number of CFUs counted in each of the fifteen 10 µL dots for each 12-hour time sampling is reported. While all fifteen 10 µL dots are technical replicates, they were divided into three groups of five for easier data analysis where Group 1, Group 2, and Group 3 are color coded blue, orange, and green respectively for each biological replicate.

Table A10: Data Analysis of Biological Replicate 1

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	3	4.4	3.4	3.00E6	4.40E6	3.40E6	6.477	6.643	6.531	6.551
0.5	15.2	13	14.2	1.52E9	1.30E9	1.42E9	9.182	9.114	9.152	9.149
1	13.8	13.2	14.2	1.38E9	1.32E9	1.42E9	9.140	9.121	9.152	9.138
1.5	12.4	10.6	10	1.24E9	1.06E9	1.00E9	9.093	9.025	9.000	9.040
2	17.4	18	19.4	1.74E9	1.80E9	1.94E9	9.241	9.255	9.288	9.261
2.5	21.6	22.4	21.2	2.16E9	2.24E9	2.12E9	9.334	9.350	9.326	9.337
3	29.2	23.8	21	2.92E9	2.38E9	2.10E9	9.465	9.377	9.322	9.388

Table A11: Data Analysis of Biological Replicate 2

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	2.8	5	3	2.80E6	5.00E6	3.00E6	6.447	6.699	6.477	6.541
0.5	5	6.8	4.6	5.00E8	6.80E8	4.60E8	8.699	8.833	8.663	8.731
1	20.6	21	23	2.06E9	2.10E9	2.30E9	9.314	9.322	9.362	9.333
1.5	22	19.6	21.2	2.20E9	1.96E9	2.12E9	9.342	9.292	9.326	9.320
2	17.6	15.6	19	1.76E9	1.56E9	1.90E9	9.246	9.193	9.279	9.239
2.5	55.4	56.4	57.2	5.54E9	5.64E9	5.72E9	9.744	9.751	9.757	9.751
3	32.8	32	33.8	3.28E9	3.20E9	3.38E9	9.516	9.505	9.529	9.517

Table A12: Data Analysis of Biological Replicate 3

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	2	3.8	3	2.00E6	3.80E6	3.00E6	6.301	6.580	6.477	6.453
0.5	4.2	6.6	4.8	4.20E8	6.60E8	4.80E8	8.623	8.820	8.681	8.708
1	27	24.8	28.4	2.70E9	2.48E9	2.84E9	9.431	9.394	9.453	9.426
1.5	14.2	13.6	14	1.42E9	1.36E9	1.40E9	9.152	9.134	9.146	9.144
2	17.4	16.4	16.4	1.74E9	1.64E9	1.64E9	9.241	9.215	9.215	9.223
2.5	26.8	30.6	29	2.68E9	3.06E9	2.90E9	9.428	9.486	9.462	9.459
3	30.2	32.2	23.4	3.02E9	3.22E9	2.34E9	9.480	9.508	9.369	9.452

Tables A10-A12: Tables A10, A11, and, A12 show further analysis of the data collected in Tables A7, A8, and A9 respectively. The average CFU from each group in Tables A7, A8, and A9 were calculated. This average CFU number was then back calculated to CFU/mL. The Log_{10} (CFU/mL) for each group was calculated, and an average Log_{10} (CFU/mL) from all group is given in the final column.

Results of Wild-Type Individual Growth Curve in Nutrient Poor (MAM) Media

Table A13: Biological Replicate 1

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.12	4	24	14	16	19	22	19	18	18	16	20	24	30	19	18	15
1	3.65	6	117	123	107	78	107	93	110	63	106	117	129	110	95	122	123
2	8.58	6	29	33	42	27	32	28	30	25	20	26	34	31	32	29	33
3	8.24	6	33	34	36	24	35	32	27	31	35	46	32	26	36	35	41
5	7.66	5	16	7	17	19	11	8	16	13	15	14	13	8	10	17	12
7	8.44	5	10	9	7	5	11	10	11	12	14	10	11	11	14	8	8

Table A14: Biological Replicate 2

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.08	4	20	34	32	36	27	26	23	24	20	14	23	16	24	24	26
1	4.30	6	94	120	113	118	105	118	133	106	100	113	125	131	143	124	104
2	9.85	6	46	41	43	37	33	32	33	41	35	36	39	30	36	31	43
3	9.95	6	27	29	24	25	24	28	26	27	26	20	29	24	23	25	18
5	7.52	5	42	43	37	41	35	37	39	35	39	42	43	52	54	44	56
7	8.21	5	8	9	6	7	11	10	8	5	5	8	6	9	11	12	10

Table A15: Biological Replicate 3

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.08	4	24	24	21	22	17	21	21	21	25	20	25	24	21	21	19
1	5.10	6	102	92	99	112	94	104	111	114	84	109	98	66	91	112	107
2	8.01	6	34	43	40	38	38	40	38	38	39	44	38	36	33	48	42
3	8.04	6	23	25	20	20	22	21	26	22	22	21	24	15	26	17	20
5	7.95	5	7	6	6	6	9	5	6	6	5	11	6	9	8	4	5
7	8.56	5	6	11	10	10	10	5	6	4	6	5	5	8	5	6	5

Tables A13-A15: These tables show direct data gathered from each of the three nutrient poor media wild-type *M. luteus* biological replicates every 24-48 hours over the course of seven days. Table A13, A14, and A15 are the biological replicates 1, 2, and 3 respectively. The optical density, serial dilution, and number of CFUs counted in each of the fifteen 10 µL dots for each time sampling is reported. While all fifteen 10 µL dots are technical replicates, they were divided into three groups of five for easier data analysis where Group 1, Group 2, and Group 3 are color coated blue, orange, and green respectively for each biological replicate.

Table A16: Data Analysis of Biological Replicate 1

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	19	18.2	21.2	1.90E7	1.82E7	2.12E7	7.279	7.260	7.326	7.288
1	106.4	97.8	115.8	1.06E10	9.78E9	1.16E10	10.027	9.990	10.064	10.027
2	32.6	25.8	31.8	3.26E9	2.58E9	3.18E9	9.513	9.412	9.502	9.476
3	32.4	34.2	34	3.24E9	3.42E9	3.40E9	9.511	9.534	9.531	9.525
5	14	13.2	12	1.40E8	1.32E8	1.20E8	8.146	8.121	8.079	8.115
7	8.4	11.4	10.4	8.40E7	1.14E8	1.04E8	7.924	8.057	8.017	7.999

Table A17: Data Analysis of Biological Replicate 2

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	29.8	21.4	22.6	2.98E7	2.14E7	2.26E7	7.474	7.330	7.354	7.386
1	110	114	125.4	1.10E10	1.14E10	1.25E10	10.041	10.057	10.098	10.066
2	40	35.4	35.8	4.00E9	3.54E9	3.58E9	9.602	9.549	9.554	9.568
3	25.8	25.4	23.8	2.58E9	2.54E9	2.38E9	9.412	9.405	9.377	9.398
5	39.6	38.4	49.8	3.96E8	3.84E8	4.98E8	8.598	8.584	8.697	8.626
7	8.2	7.2	9.6	8.20E7	7.20E7	9.60E7	7.914	7.857	7.982	7.918

Table A18: Data Analysis of Biological Replicate 3

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	21.6	21.6	22	2.16E7	2.16E7	2.20E7	7.334	7.334	7.342	7.337
1	99.8	104.4	94.8	9.98E9	1.04E10	9.48E9	9.999	10.019	9.977	9.998
2	38.6	39.8	39.4	3.86E9	3.98E9	3.94E9	9.587	9.600	9.595	9.594
3	22	22.4	20.4	2.20E9	2.24E9	2.04E9	9.342	9.350	9.310	9.334
5	6.8	6.6	6.4	6.80E7	6.60E7	6.40E7	7.833	7.820	7.806	7.819
7	9.4	5.2	5.8	9.40E7	5.20E7	5.80E7	7.973	7.716	7.763	7.818

Tables A16-A18: Tables A16, A17, and A18 show further analysis of the data collected in Tables A13, A14, and A15 respectively. The average CFU from each group in Tables A13, A14, and A15 were calculated. This average CFU number was then back calculated to CFU/mL. The Log_{10} (CFU/mL) for each group was calculated, and an average Log_{10} (CFU/mL) of all group is given in the final column.

Results of *ΔcrtE*::kan Individual Growth Curve in Nutrient Poor (MAM) Media

Table A19: Biological Replicate 1

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.06	3	17	14	25	27	20	18	28	18	18	25	15	25	16	23	12
1	5.48	6	46	60	51	56	48	49	47	57	53	61	63	52	40	56	61
2	6.18	6	18	12	18	27	10	12	18	22	14	21	20	17	15	19	15
3	6.61	6	29	28	33	30	37	36	31	32	29	32	32	35	29	32	34
5	6.04	4	48	54	59	51	57	50	52	66	57	50	62	56	52	59	64
7	5.90	4	13	9	7	10	8	10	9	10	9	9	12	9	9	6	7

Table A20: Biological Replicate 2

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.067	3	30	33	29	27	33	34	33	29	33	33	28	26	23	35	34
1	6.15	6	59	67	52	60	58	59	61	64	62	62	52	51	72	59	53
2	7.03	6	21	19	27	20	21	19	19	22	26	26	24	21	23	22	26
3	6.88	6	21	13	18	13	9	12	16	10	14	5	14	7	8	11	12
5	6.40	4	34	25	36	37	27	36	31	34	39	35	35	30	36	26	42
7	6.89	4	13	14	14	19	11	11	11	14	18	9	15	9	7	11	13

Table A21: Biological Replicate 3

Time Days	OD_{600}	Dil.	Dot Replicates (10 µL each)														
			Group 1					Group 2					Group 3				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	0.063	3	27	25	24	28	28	31	29	24	27	20	26	22	20	23	27
1	5.51	6	60	72	71	78	78	71	81	66	77	68	61	88	75	81	65
2	6.95	6	22	26	24	18	18	23	21	23	18	17	20	21	20	25	25
3	6.63	6	9	18	15	14	15	18	14	14	15	15	20	18	21	17	13
5	6.50	4	28	29	34	33	31	41	33	28	28	29	34	27	33	25	29
7	6.55	4	5	14	10	14	16	18	12	13	17	12	22	13	15	13	14

Tables A19-A21: These tables show direct data gathered from each of the three nutrient poor media *Δcrt*E::kan *M. luteus* biological replicates every 24-48 hours over the course of seven days. Table A19, A20, and A21 are the biological replicates 1, 2, and 3 respectively. The optical density, serial dilution, and number of CFUs counted in each of the fifteen 10 µL dots for each time sampling is reported. While all fifteen 10 µL dots are technical replicates, they were divided into three groups of five for easier data analysis where Group 1, Group 2, and Group 3 are color coated blue, orange, and green respectively for each biological replicate.

Table A22: Data Analysis of Biological Replicate 1

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	20.6	21.4	18.2	2.06E6	2.14E6	1.82E6	6.314	6.330	6.260	6.301
1	52.2	53.4	54.4	5.22E9	5.34E9	5.44E9	9.718	9.728	9.736	9.727
2	17	17.4	17.2	1.70E9	1.74E9	1.72E9	9.230	9.241	9.236	9.236
3	31.4	32	32.4	3.14E9	3.20E9	3.24E9	9.497	9.505	9.511	9.504
5	53.8	55	58.6	5.38E7	5.50E7	5.86E7	7.731	7.740	7.768	7.746
7	9.4	9.4	8.6	9.40E6	9.40E6	8.60E6	6.973	6.973	6.934	6.960

Table A23: Data Analysis of Biological Replicate 2

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	30.4	32.4	29.2	3.04E6	3.24E6	2.92E6	6.483	6.511	6.465	6.486
1	59.2	61.6	57.4	5.92E9	6.16E9	5.74E9	9.772	9.790	9.759	9.774
2	21.6	22.4	23.2	2.16E9	2.24E9	2.32E9	9.334	9.350	9.365	9.350
3	14.8	11.4	10.4	1.48E9	1.14E9	1.04E9	9.170	9.057	9.017	9.081
5	31.8	35	33.8	3.18E7	3.50E7	3.38E7	7.502	7.544	7.529	7.525
7	14.2	12.6	11	1.42E7	1.26E7	1.10E7	7.152	7.100	7.041	7.098

Table A24: Data Analysis of Biological Replicate 3

Time Days	Avg Grp 1	Avg Grp 2	Avg Grp 3	CFU/mL Grp 1	CFU/mL Grp 2	CFU/mL Grp 3	Log CFU/mL Grp 1	Log CFU/mL Grp 2	Log CFU/mL Grp 3	Average Log CFU/mL
0	26.4	26.2	23.6	2.64E6	2.62E6	2.36E6	6.422	6.418	6.373	6.404
1	71.8	72.6	74	7.18E9	7.26E9	7.40E9	9.856	9.861	9.869	9.862
2	21.6	20.4	22.2	2.16E9	2.04E9	2.22E9	9.334	9.310	9.346	9.330
3	14.2	15.2	17.8	1.42E9	1.52E9	1.78E9	9.152	9.182	9.250	9.195
5	31	31.8	29.6	3.10E7	3.18E7	2.96E7	7.491	7.502	7.471	7.488
7	11.8	14.4	15.4	1.18E7	1.44E7	1.54E7	7.072	7.158	7.188	7.139

Tables A22-A24: Tables A22, A23, and, A24 show further analysis of the data collected in Tables A19, A20, and A21 respectively. The average CFU from each group in Tables A19, A20, and A21 were calculated. This average CFU number was then back calculated to CFU/mL. The Log_{10} (CFU/mL) for each group was calculated, and an average Log_{10} (CFU/mL) of all group is given in the final column.

WT versus *ΔcrtE*::kan Competition Experiments in mNBE Media

Table A25: Biological Replicate 1

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio
0	0.023	3	57	56	113	0.504	0.495	62	64	126	0.492	0.507	77	73	150	0.513	0.487
0.5	0.72	5	58	51	109	0.532	0.467	46	47	93	0.494	0.505	35	42	77	0.455	0.545
1	5.45	7	99	115	214	0.462	0.537	132	119	251	0.525	0.474	145	127	272	0.533	0.467
1.5	6.33	7	20	17	37	0.540	0.459	13	24	37	0.351	0.648	14	17	31	0.452	0.548
2	6.02	6	24	33	57	0.421	0.578	24	27	51	0.470	0.529	30	44	74	0.405	0.595
2.5	5.62	7	130	146	276	0.471	0.528	290	356	646	0.448	0.551	216	271	487	0.444	0.556
3	5.29	6	107	88	195	0.548	0.451	66	61	127	0.519	0.480	39	30	69	0.565	0.435

Table A26: Biological Replicate 2

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio
0	0.029	3	64	34	98	0.653	0.347	48	43	91	0.527	0.473	51	65	116	0.440	0.560
0.5	0.76	5	35	47	82	0.427	0.573	30	30	60	0.500	0.500	22	40	62	0.355	0.645
1	5.03	7	33	22	55	0.600	0.400	30	29	59	0.508	0.492	10	14	24	0.417	0.583
1.5	6.22	7	20	14	34	0.588	0.412	12	18	30	0.400	0.600	14	18	32	0.438	0.563
2	6.0	6	39	35	74	0.527	0.473	55	51	106	0.519	0.481	67	50	117	0.573	0.427
2.5	5.58	7	57	52	109	0.523	0.477	146	152	298	0.490	0.510	93	104	197	0.472	0.528
3	5.30	6	21	14	35	0.600	0.400	32	25	57	0.561	0.439	26	33	59	0.441	0.559

Table A27: Biological Replicate 3

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio	White CFU	Yellow CFU	Total CFU	CrtE Ratio	WT Ratio
0	0.023	3	132	144	276	0.478	0.522	110	99	209	0.526	0.474	119	86	205	0.580	0.420
0.5	0.44	5	10	16	26	0.385	0.615	17	15	32	0.531	0.469	29	26	55	0.527	0.473
1	4.13	7	14	21	35	0.400	0.600	20	21	41	0.488	0.512	25	21	46	0.543	0.457
1.5	6.21	7	18	21	39	0.462	0.538	18	24	42	0.429	0.571	29	16	45	0.644	0.356
2	6.16	6	32	27	59	0.542	0.458	48	33	81	0.593	0.407	29	28	57	0.509	0.491
2.5	5.71	7	61	45	106	0.575	0.425	55	42	97	0.567	0.433	48	54	102	0.471	0.529
3	5.32	6	44	27	71	0.620	0.380	52	39	91	0.571	0.429	40	43	83	0.482	0.518

Table A28: Biological Replicate 4

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio
0	0.02	3	46	57	103	0.447	0.553	33	33	66	0.500	0.500	34	45	79	0.430	0.570
0.5	0.60	5	62	46	108	0.574	0.426	41	55	96	0.427	0.573	40	53	93	0.430	0.570
1	3.41	6	171	106	277	0.617	0.383	172	116	288	0.597	0.403	180	147	327	0.550	0.450
1.5	5.91	7	34	38	72	0.472	0.528	50	41	91	0.549	0.451	76	63	139	0.547	0.453
2	5.51	6	13	5	18	0.722	0.278	22	22	44	0.500	0.500	10	11	21	0.476	0.524
2.5	5.13	6	56	56	112	0.500	0.500	93	50	143	0.650	0.350	55	33	88	0.625	0.375
3	4.63	6	82	68	150	0.547	0.453	44	38	82	0.537	0.463	70	46	116	0.603	0.397

Tables A25-A28: These tables show the number of white *ΔuspA616*::kan (*ΔcrtE*) and yellow wild-type (WT) colonies, the total number of CFUs and the ratio of *ΔcrtE* and WT CFUs for the three plates (technical replicates) for each of the four biological replicates for each time sampling over the course of three days.

Table A29: Data Analysis of Biological Replicate 1

Time (Days)	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.756	5.748	5.792	5.806	5.886	5.863
0.5	7.763	7.708	7.663	7.672	7.544	7.623
1	9.996	10.061	10.121	10.076	10.161	10.104
1.5	9.301	9.230	9.114	9.380	9.146	9.230
2	8.380	8.519	8.380	8.431	8.477	8.643
2.5	10.114	10.164	10.462	10.551	10.334	10.433
3	9.029	8.944	8.820	8.785	8.591	8.477

Table A30: Data Analysis of Biological Replicate 2

Time (Days)	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.806	5.531	5.681	5.633	5.708	5.813
0.5	7.544	7.672	7.477	7.477	7.342	7.602
1	9.519	9.342	9.477	9.462	9.000	9.146
1.5	9.301	9.146	9.079	9.255	9.146	9.255
2	8.591	8.544	8.740	8.708	8.826	8.699
2.5	9.756	9.716	10.164	10.182	9.968	10.017
3	8.322	8.146	8.505	8.398	8.415	8.519

Table A31: Data Analysis of Biological Replicate 3

Time (Days)	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	6.121	6.158	6.041	5.996	6.076	5.934
0.5	7.000	7.204	7.230	7.176	7.462	7.415
1	9.146	9.322	9.301	9.322	9.398	9.322
1.5	9.255	9.322	9.255	9.380	9.462	9.204
2	8.505	8.431	8.681	8.519	8.462	8.447
2.5	9.785	9.653	9.740	9.623	9.681	9.732
3	8.643	8.431	8.716	8.591	8.602	8.633

Table A32: Data Analysis of Biological Replicate 4

Time (Days)	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.663	5.756	5.519	5.519	5.531	5.653
0.5	7.792	7.663	7.613	7.740	7.602	7.724
1	9.233	9.025	9.236	9.064	9.255	9.167
1.5	9.531	9.580	9.699	9.613	9.881	9.799
2	8.114	7.699	8.342	8.342	8.000	8.041
2.5	8.748	8.748	8.968	8.699	8.740	8.519
3	8.914	8.833	8.643	8.580	8.845	8.663

Tables A29-A32: These tables show the Log_{10} (CFU/mL) of the *ΔcrtE* and WT colonies for the three plates for the four biological replicates for each sampling time.

WT versus *ΔcrtE*::kan Competition Experiments in Minimal Acetate Media

Table A33: Biological Replicate 1

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio
0	0.041	3	48	55	103	0.466	0.534	61	65	126	0.484	0.516	76	80	156	0.487	0.513
1	3.89	5	260	244	504	0.516	0.484	296	226	522	0.567	0.433	268	212	480	0.558	0.442
2	4.26	6	16	16	32	0.500	0.500	27	10	37	0.730	0.270	23	18	41	0.561	0.439
3	4.48	6	34	15	49	0.694	0.306	32	19	51	0.627	0.373	36	16	52	0.692	0.308
5	3.61	5	346	230	576	0.601	0.399	296	183	479	0.618	0.382	252	141	393	0.641	0.359
7	3.25	5	39	23	62	0.629	0.371	44	18	62	0.710	0.290	24	20	44	0.545	0.455

Table A34: Biological Replicate 2

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio
0	0.040	3	89	65	154	0.578	0.422	51	54	105	0.486	0.514	64	77	141	0.454	0.546
1	3.79	5	154	75	229	0.672	0.328	134	76	210	0.638	0.362	111	84	195	0.569	0.431
2	4.09	6	66	45	111	0.595	0.405	62	37	99	0.626	0.374	69	36	105	0.657	0.343
3	3.86	6	18	5	23	0.783	0.217	21	12	33	0.636	0.364	18	9	27	0.667	0.333
5	3.53	5	158	94	252	0.627	0.373	114	66	180	0.633	0.367	148	85	233	0.635	0.365
7	3.30	5	33	15	48	0.688	0.313	31	6	37	0.838	0.162	34	16	50	0.680	0.320

Table A35: Biological Replicate 3

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio
0	0.041	3	57	31	88	0.648	0.352	55	35	90	0.611	0.389	52	44	96	0.542	0.458
1	3.23	5	91	62	153	0.595	0.405	103	66	169	0.609	0.391	95	59	154	0.617	0.383
2	3.29	6	39	48	87	0.448	0.552	48	33	81	0.593	0.407	37	41	78	0.474	0.526
3	3.16	6	25	18	43	0.581	0.419	18	24	42	0.429	0.571	27	17	44	0.614	0.386
5	3.15	5	716	546	1262	0.567	0.433	690	506	1196	0.577	0.423	493	379	872	0.565	0.435
7	2.91	5	131	71	202	0.649	0.351	125	55	180	0.694	0.306	109	49	158	0.690	0.310

Table A36: Biological Replicate 4

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	WT Ratio
0	0.038	3	66	67	133	0.496	0.504	74	71	145	0.510	0.490	90	68	158	0.570	0.430
1	3.44	5	79	44	123	0.642	0.358	93	53	146	0.637	0.363	99	56	155	0.639	0.361
2	3.79	6	36	25	61	0.590	0.410	57	32	89	0.640	0.360	19	18	37	0.514	0.486
3	3.33	6	68	51	119	0.571	0.429	61	23	84	0.726	0.274	46	33	79	0.582	0.418
5	3.39	5	216	149	365	0.592	0.408	215	134	349	0.616	0.384	209	115	324	0.645	0.355
7	3.04	5	40	26	66	0.606	0.394	59	25	84	0.702	0.298	52	16	68	0.765	0.235

Table A33-A36: These tables show the number of white (*ΔcrtE*) and yellow (WT) colonies, the total number of CFUs and the ratio of *ΔcrtE* and WT CFUs of the three plates (technical replicates) for each of the four biological replicates for each time sampling over the course of seven days.

Table A37: Data Analysis of Biological Replicate 1

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.681	5.740	5.785	5.813	5.881	5.903
1	8.415	8.387	8.471	8.354	8.428	8.326
2	8.204	8.204	8.431	8.000	8.362	8.255
3	8.531	8.176	8.505	8.279	8.556	8.204
5	8.539	8.362	8.471	8.262	8.401	8.149
7	7.591	7.362	7.643	7.255	7.380	7.301

Table A38: Data Analysis of Biological Replicate 2

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.949	5.813	5.708	5.732	5.806	5.886
1	8.188	7.875	8.127	7.881	8.045	7.924
2	8.820	8.653	8.792	8.568	8.839	8.556
3	8.255	7.699	8.322	8.079	8.255	7.954
5	8.199	7.973	8.057	7.820	8.170	7.929
7	7.519	7.176	7.491	6.778	7.531	7.204

Table A39: Data Analysis of Biological Replicate 3

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.756	5.491	5.740	5.544	5.716	5.643
1	7.959	7.792	8.013	7.820	7.978	7.771
2	8.591	8.681	8.681	8.519	8.568	8.613
3	8.398	8.255	8.255	8.380	8.431	8.230
5	8.855	8.737	8.839	8.704	8.693	8.579
7	8.117	7.851	8.097	7.740	8.037	7.690

Table A40: Data Analysis of Biological Replicate 4

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	5.820	5.826	5.869	5.851	5.954	5.833
1	7.898	7.643	7.968	7.724	7.996	7.748
2	8.556	8.398	8.756	8.505	8.279	8.255
3	8.833	8.708	8.785	8.362	8.663	8.519
5	8.334	8.173	8.332	8.127	8.320	8.061
7	7.602	7.415	7.771	7.398	7.716	7.204

Tables A37-A40: These tables show the Log_{10} (CFU/mL) of the *ΔcrtE* and WT colonies for the three plates for the four biological replicates for each sampling time.

ΔcrtE::kan versus *ΔuspA616*::kan Competition Experiments in mNBE Media

Table A41: Biological Replicate 1

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio
0	0.051	3	158	149	307	0.515	0.485	177	122	299	0.592	0.408	136	120	256	0.531	0.469
0.5	2.75	4	49	45	94	0.521	0.479	40	33	73	0.548	0.452	58	40	98	0.592	0.408
1	6.83	5	90	67	157	0.573	0.427	68	44	112	0.607	0.393	73	44	117	0.624	0.376
1.5	7.08	6	49	29	78	0.628	0.372	26	21	47	0.553	0.447	42	34	76	0.553	0.447
2	6.61	6	48	27	75	0.640	0.360	26	20	46	0.565	0.435	12	10	22	0.545	0.455
2.5	6.51	6	127	115	242	0.525	0.475	121	98	219	0.553	0.447	119	98	217	0.548	0.452
3	6.33	5	227	174	401	0.566	0.434	263	178	441	0.596	0.404	191	149	340	0.562	0.438

Table A42: Biological Replicate 2

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio
0	0.043	3	147	103	250	0.588	0.412	131	79	210	0.624	0.376	135	103	238	0.567	0.433
0.5	3.10	4	84	53	137	0.613	0.387	61	31	92	0.663	0.337	40	24	64	0.625	0.375
1	6.74	5	84	69	153	0.549	0.451	89	49	138	0.645	0.355	77	62	139	0.554	0.446
1.5	7.06	6	73	32	105	0.695	0.305	35	20	55	0.636	0.364	28	15	43	0.651	0.349
2	6.66	6	69	43	112	0.616	0.384	34	14	48	0.708	0.292	43	18	61	0.705	0.295
2.5	6.13	6	154	103	257	0.599	0.401	158	117	275	0.575	0.425	167	107	274	0.609	0.391
3	5.93	5	335	231	566	0.592	0.408	275	156	431	0.638	0.362	222	137	359	0.618	0.382

Table A43: Biological Replicate 3

Time Days	OD_{600}	Dil.	Plate 1					Plate 2					Plate 3				
			White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio	White CFUs	Yellow CFUs	Total CFUs	CrtE Ratio	Δ616 Ratio
0	0.048	3	173	109	282	0.613	0.387	196	163	359	0.546	0.454	140	111	251	0.558	0.442
0.5	3.77	4	62	55	117	0.530	0.470	54	42	96	0.563	0.438	31	22	53	0.585	0.415
1	7.98	5	166	106	272	0.610	0.390	94	67	161	0.584	0.416	99	72	171	0.579	0.421
1.5	7.89	6	46	32	78	0.590	0.410	46	59	105	0.438	0.562	36	25	61	0.590	0.410
2	7.30	6	41	36	77	0.532	0.468	28	15	43	0.651	0.349	39	35	74	0.527	0.473
2.5	7.10	6	66	70	136	0.485	0.515	65	43	108	0.602	0.398	77	59	136	0.566	0.434
3	7.18	5	573	379	952	0.602	0.398	365	220	585	0.624	0.376	309	119	428	0.722	0.278

Tables A41-A43: These tables show the number of white (*ΔcrtE*) and yellow (*ΔuspA616*) colonies, the total number of CFUs and the ratio of *ΔcrtE* and *ΔuspA616* CFUs for the three plates (technical replicates) for each of the three biological replicates for each time sampling over the course of three days.

Table A44: Data Analysis of Biological Replicate 1

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	6.199	6.173	6.248	6.086	6.134	6.079
0.5	6.690	6.653	6.602	6.519	6.763	6.602
1	7.954	7.826	7.833	7.643	7.863	7.643
1.5	8.690	8.462	8.415	8.322	8.623	8.531
2	8.681	8.431	8.415	8.301	8.079	8.000
2.5	9.104	9.061	9.083	8.991	9.076	8.991
3	8.356	8.241	8.420	8.250	8.281	8.173

Table A45: Data Analysis of Biological Replicate 2

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	6.167	6.013	6.117	5.898	6.130	6.013
0.5	6.924	6.724	6.785	6.491	6.602	6.380
1	7.924	7.839	7.949	7.690	7.886	7.792
1.5	8.863	8.505	8.544	8.301	8.447	8.176
2	8.839	8.633	8.531	8.146	8.633	8.255
2.5	9.188	9.013	9.199	9.068	9.223	9.029
3	8.525	8.364	8.439	8.193	8.346	8.137

Table A46: Data Analysis of Biological Replicate 3

Time Days	Plate 1		Plate 2		Plate 3	
	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)	White Log_{10} (CFU/mL)	Yellow Log_{10} (CFU/mL)
0	6.238	6.037	6.292	6.212	6.146	6.045
0.5	6.792	6.740	6.732	6.623	6.491	6.342
1	8.220	8.025	7.973	7.826	7.996	7.857
1.5	8.663	8.505	8.663	8.771	8.556	8.398
2	8.613	8.556	8.447	8.176	8.591	8.544
2.5	8.820	8.845	8.813	8.633	8.886	8.771
3	8.758	8.579	8.562	8.342	8.490	8.076

Tables A44-A46: These tables show the Log_{10} (CFU/mL) of *ΔcrtE* and *ΔuspA616* colonies for the three plates for the three biological replicates for each sampling time.

ΔcrtE::kan versus *ΔuspA616*::kan Competition Experiments in Minimal Acetate Media

Table A47: Biological Replicate 1

Time Days	Dil.	Dot Replicates (20 µL each)												Average Yellow CFU	Average White CFU	Average Sum
		Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6				
		Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU			
0	3	13	20	13	13	18	16	15	13	10	17	11	13	13.333	15.333	28.667
2	5	2	3	1	0	2	0	3	1	1	0	0	2	1.500	1.000	2.500
4	6	20	0	10	2	13	2	8	3	16	4	14	2	13.500	2.167	15.667
6	5	0	7	0	1	0	2	0	3	0	3	0	3	0.000	3.167	3.167
8	5	0	1	0	0	0	4	0	2	0	2	0	1	0.000	1.667	1.667
10	3	1	2	0	3	0	3	0	4	0	2	0	2	0.167	2.667	2.833
12	3	0	1	0	3	0	1	0	4	3	21	0	0	0.500	5.000	5.500
14	3	0	4	0	4	0	2	0	11	0	5	0	3	0.000	4.833	4.833

Table A48: Biological Replicate 2

Time Days	Dil.	Dot Replicates (20 µL each)												Average Yellow CFU	Average White CFU	Average Sum
		Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6				
		Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU			
0	3	11	18	10	16	14	16	18	14	10	12	11	13	12.333	14.833	27.167
2	5	6	0	2	1	3	2	2	2	4	6	4	3	3.500	2.333	5.833
4	5	13	3	12	5	9	4	12	5	13	2	17	3	12.667	3.667	16.333
6	5	0	6	0	10	0	6	1	6	0	4	0	8	0.167	6.667	6.833
8	5	0	13	0	11	1	12	0	9	0	7	0	6	0.167	9.667	9.833
10	4	0	33	0	37	0	33	0	42	0	30	0	42	0.000	36.167	36.167
12	4	0	30	0	26	0	37	0	30	0	27	0	32	0.000	30.333	30.333
14	4	0	26	0	17	0	24	0	27	0	35	0	22	0.000	25.167	25.167

Table A49: Biological Replicate 3

Time Days	Dil.	Dot Replicates (20 µL each)												Average Yellow CFU	Average White CFU	Average Sum
		Replicate 1		Replicate 2		Replicate 3		Replicate 4		Replicate 5		Replicate 6				
		Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU	Yellow CFU	White CFU			
0	3	11	15	9	16	10	13	10	13	16	14	13	8	11.500	13.167	24.667
2	5	24	8	20	5	15	5	12	4	21	7	21	5	18.833	5.667	24.500
4	5	17	22	6	25	16	15	16	25	5	24	11	29	11.833	23.333	35.167
6	5	0	14	0	8	0	16	0	17	0	11	0	16	0.000	13.667	13.667
8	5	0	1	0	4	0	1	0	2	0	1	0	1	0.000	1.667	1.667
10	3	0	28	0	23	0	22	0	16	0	21	0	23	0.000	22.167	22.167
12	3	0	20	0	25	0	23	0	29	0	24	0	23	0.000	24.000	24.000
14	3	0	65	1	66	0	61	0	57	0	65	0	63	0.167	62.833	63.000

Tables A47-A49: These tables show the number of yellow (*ΔuspA616*) and white (*ΔcrtE*) CFUs in the six technical replicate 20 µL dots with the different dilutions and time of sampling for the three biological replicates. An average yellow, average white, and average sum CFU number is also presented for each sampling time in the three biological replicates.

Table A50: Data Analysis of Biological Replicate 1-3

Time Days	Biological Replicate 1				Biological Replicate 2				Biological Replicate 3			
	Yellow CFU/mL	White CFU/mL	Log_{10} Yellow (CFU/mL)	Log_{10} White (CFU/mL)	Yellow CFU/mL	White CFU/mL	Log_{10} Yellow (CFU/mL)	Log_{10} White (CFU/mL)	Yellow CFU/mL	White CFU/mL	Log_{10} Yellow (CFU/mL)	Log_{10} White (CFU/mL)
0	6.66E5	7.66E5	5.824	5.885	6.16E5	7.42E5	5.790	5.870	5.75E5	6.58E5	5.760	5.818
2	7.50E6	5.00E6	6.875	6.699	1.75E7	1.17E7	7.243	7.067	9.42E7	2.83E7	7.974	7.452
4	6.75E8	1.08E8	8.829	8.035	6.33E7	1.83E7	7.802	7.263	5.92E7	1.16E8	7.772	8.067
6	0.0	1.58E7	-0.046	7.200	8.33E5	3.33E7	5.921	7.523	0.0	6.83E7	-0.046	7.835
8	0.0	8.33E6	-0.046	6.921	8.33E5	4.83E7	5.921	7.684	0.0	8.33E6	-0.046	6.921
10	8.33E3	1.33E5	3.921	5.125	0.0	1.81E7	-0.046	7.257	0.0	1.11E6	-0.046	6.045
12	2.50E4	2.50E5	4.398	5.398	0.0	1.52E7	-0.046	7.181	0.0	1.20E6	-0.046	6.079
14	0.0	2.42E5	-0.046	5.383	0.0	1.26E7	-0.046	7.100	8.33E3	3.14E6	3.921	6.497

Table A50: This table presents the average yellow (*ΔuspA616*) CFU/mL and average white (*ΔcrtE*) CFU/mL for each biological replicate. This was calculated from data shown in Tables A47-A49. The Log_{10} of the CFU/mL was also calculated and shown for each biological replicate.

Table A51: Ratio Data of Biological Replicates 1-3

Time (Days)	Biological Replicate 1		Biological Replicate 2		Biological Replicate 3	
	ΔuspA616 Ratio	*ΔcrtE* Ratio	*ΔuspA616* Ratio	*ΔcrtE* Ratio	*ΔuspA616* Ratio	*ΔcrtE* Ratio
0	0.465	0.535	0.454	0.546	0.466	0.534
2	0.600	0.400	0.600	0.400	0.769	0.231
4	0.862	0.138	0.776	0.224	0.336	0.664
6	0.000	1.000	0.024	0.976	0.000	1.000
8	0.000	1.000	0.017	0.983	0.000	1.000
10	0.059	0.941	0.000	1.000	0.000	1.000
12	0.091	0.909	0.000	1.000	0.000	1.000
14	0.000	1.000	0.000	1.000	0.003	0.997

Table A51: This table presents the average yellow (*ΔuspA616*) and white (*ΔcrtE*) CFU ratios calculated from average yellow and white CFU data shown in Tables A47-A49.