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DYNAMICS ASSOCIATED WITH BACTERIOCIN RESISTANCE IN PSEUDOMONAS SYRINGAE

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ABSTRACT

Pseudomonas syringae pv. *actinidiae* (*Psa* BV3) has caused severe economic damage the New Zealand kiwifruit industry following its introduction in 2010. A related strain, *Pseudomonas syringae* pv. *syringae* B728a (*Psy* B728a), produces two toxins, an R-type (retractile, phage derived) and an S-type (soluble) bacteriocin, that are active against *Psa* BV3. These target *Psa* BV3 in a 'conditionally redundant' way, preventing it from developing resistance to the R-type bacteriocin while in the presence of the S-type, a system which could be used as a biocontrol strategy. To increase knowledge of this system and evaluate its biocontrol potential, this project focused on finding the gene responsible for developing S-type resistance and determining if there was a fitness cost incurred by this resistance. Twelve R- and S-type resistant mutants were developed, sequenced, and aligned with their parent strain to observe the genomic and genetic changes that had occurred. An area of about 70 genes was lost in 9 of the mutants, with another having a missense mutation in a gene in this region. The remaining two resistant strains had other mutated genes. This analysis identified the hemolysin secretion/activation, TonB dependent siderophore receptor, *hflB*, and PQQ-dependent catabolism-associated beta propeller genes as candidates for affecting S-type resistance. Two mutant strains had slower growth rates than the wild type while two others had growth rates that were the same. The growth rate of a slower growing strain was measured in supernatant of *Psa* BV3 and bacteriocin deactivated *Psy* B728a to observe whether normal growth was recovered since all mutants arose at the same time during generation, however no growth rate recovery was observed. This suggests that while the *Psy* B728a bacteriocin strategy effectively prevents resistance development, the lack of observed fitness cost could increase the risk involved with using this system as a biocontrol technique.

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Chapter 1

Introduction

Beginning in 2008, an outbreak of kiwifruit bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* biovar 3 (*Psa* BV3) developed and began to spread through orchards in China, Japan, Korea, and Italy (McCann et al. 2017). By 2010, the disease had entered New Zealand, where it spread rapidly due to conditions favorable for infection almost year-round as well as kiwifruit being the dominant crop in the area (Vanneste 2017). Figure 1 shows common symptoms of bacterial canker of kiwifruit.

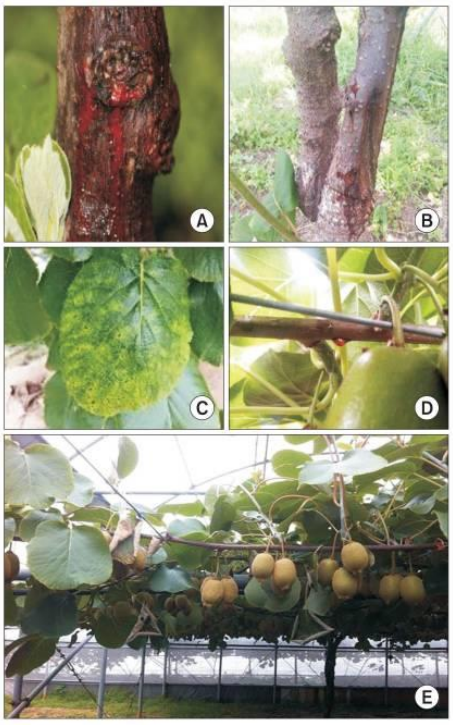

Figure 1: Symptoms of Bacterial Canker of Kiwifruit

(A and D display bacterial ooze from the trunk and a branch respectively. B shows a canker at the trunk base. C shows typical leafspots while E shows branch dieback. Image from Kim et al. 2015.)

A closely related pathogen, *Pseudomonas syringae* pv. *actinidifoliorum*, which caused similar leaf spot and bud rot symptoms and was difficult to distinguish using the available genetic tools, complicated quarantine efforts (Vanneste 2017). The resultant losses in exports between 2010 and 2014 were estimated at as high as NZ$930 million (Birdie & Livesey 2014). Considering the rapid spread of this disease worldwide and the economic implications, finding mechanisms to prevent and identify outbreaks in the future is of utmost importance.

Pseudomonas spp. are ubiquitous in the environment and can live in a variety of habitats including soil, plant, and freshwater ecosystems (Chatterjee et al. 2016). They also have complex interactions with other organisms ranging from competition with related *Pseudomonas* strains, to pathogenic interactions with plants and animals (Silby et al. 2011). *Pseudomonas* also produces a wide range of secondary metabolites functioning in nutrient acquisition, virulence, and defense against competitors and predators which can have important implications in agriculture and medicine (Gross & Loper 2009). Within this genus, *Pseudomonas syringae* represents a diverse group of plant pathogens with strains able to infect a variety of hosts as well as live epiphytically on the leaf surface (Xin et al. 2018). Multiple strains can be present on the same host, allowing for interaction with closely related competitors. Taking advantage of this genetic similarity, some strains produce receptors that can recognize compounds like siderophores produced by competitors to increase the uptake of nutrients without spending the energy necessary to produce them on their own (Hartney et al. 2013).

In order to compete with other strains in nutrient-limited environments, many strains of *Pseudomonas* produce a variety of antibacterial compounds, termed bacteriocins, which can target closely related or unrelated strains (Yang et al. 2014). These toxins often target receptors for important compounds like vitamin B12 or iron and are expressed in concert with an immunity

protein protecting the producing strain (Ghequire and Ozturk 2018). In this way, the strain can clear niche space for itself and reduce competition for resources. In these environments, competition networks can display a 'rock-paper-scissors' dynamic between the bacteriocin producer, the susceptible strain, and the resistant strain.. For instance, a bacterium produces a toxin to kill a competitor, but exerts energy to do so. If a mutation causing resistance in the targeted strain occurs, it can again compete with the producing strain. The resistant strain, however, bears the cost of losing the structure targeted by the toxin and in this way could be outcompeted when in isolation with a susceptible strain that does not bear this cost (Hibbing et al. 2015).

Pseudomonas syringae pv. *syringae* B728a (*Psy* B728a) is a related *P. syringae* strain that causes disease on varieties of beans (Vinatzer et al. 2006). *Psy* B728a produces two types of bacteriocins, R-type (retractile) and S-type (soluble), that are active against *Psa* BV3 under different circumstances. R-type bacteriocins are retractile, phage-derived toxins which have tail fibers that interact with a target, the o-antigen of the lipopolysaccharide layer in the case of *Psa* BV3 (Hockett et al. 2017). The R-type bacteriocin then punctures the cell membrane, causing loss of the electrochemical gradient across the membrane (Ghequire & DeMot 2015). These toxins function effectively like a phage without a head that allows cellular contents to leak out rather than injecting genetic material.

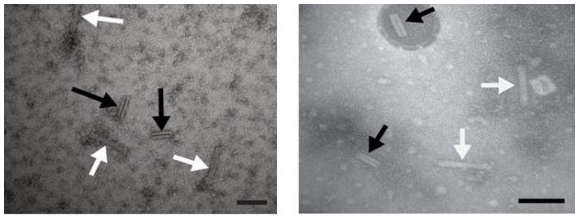

Figure 2: Electron Micrograph of R-type Bacteriocin from *Psy* B728a

(White arrows indicate complete while black arrows indicate partial components of the R-type bacteriocin. The bars at bottom right of the photos have a length of 100 nm. Image adapted from Hockett et al. 2015.)

The S-type bacteriocin is a protein which can be divided into three domains: receptor, transport, and catalytic (Hockett et al. 2017) The receptor domain recognizes a yet undetermined receptor or structure, the transport domain is responsible for passage through the cell membrane, and the catalytic domain is responsible for DNase activity which kills the target cell.

While wild type *Psa* BV3 is susceptible to the R-type bacteriocin, it is resistant to the S-type bacteriocin. Once complete resistance to the R-type bacteriocin develops, however, the strain becomes susceptible to the S-type bacteriocin. In this way, *Psy* B728a prevents the development of resistance to its toxins by creating an additional layer of selection that is only active against mutants fully resistant to the first, termed a 'conditionally redundant' toxin production strategy.

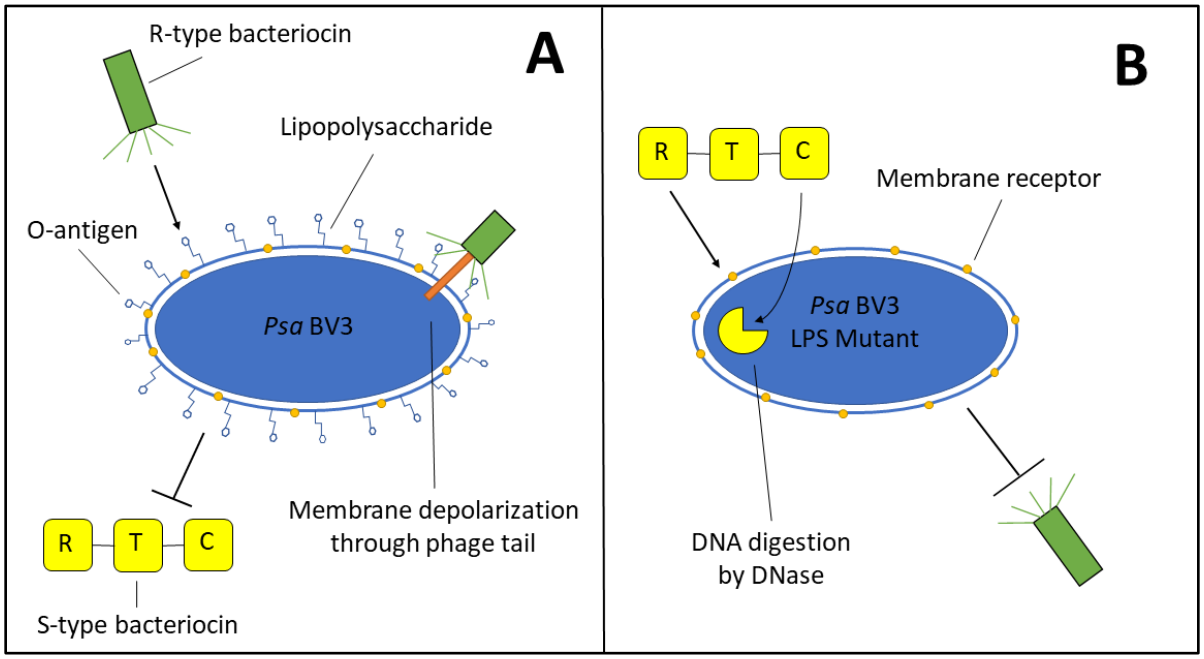

Figure 3: Conditionally Redundant Bacteriocin Production Strategy of *Psy* B728a

(Diagram depicting the behavior of *Psa* BV3 in the presence of R- and S-type bacteriocins produced by *Psy* B728a. Section A depicts wild type *Psa* BV3 which is susceptible to the R-type Bacteriocin, but not susceptible to the S-type bacteriocin. When a mutation affecting the lipopolysaccharide layer results in resistance to the R-type bacteriocin, the mutant becomes susceptible to the S-type bacteriocin, as shown in Section B.)

A strategy deploying antibacterial compounds in a conditionally redundant way could be very effective in preventing the development of resistance to these toxins. This system is most immediately applicable in biocontrols for *P. syringae*-caused disease in agriculture, but could also find application in areas like medicine, where development of antimicrobial resistance is a major concern. To progress knowledge of this system, this paper focuses on two areas of investigation. The first is determining the receptor or structure targeted by the S-type bacteriocin produced by *Psy* B728a. This is important for determining whether strains of *Psa* or closely related *P. syringae* strains can be expected to be susceptible to the S-type bacteriocin and would allow for this prediction to take place through genetic means rather than by phenotypic screening. The second area of investigation is whether mutants that are resistant to the R- and S-type bacteriocins incur a fitness cost, such as a reduced growth rate in laboratory media. This dynamic is crucial to understand before implementing this system as a biocontrol, as it is necessary to determine the consequences of the very unlikely, yet possible, chance a strain develops resistance to both toxins simultaneously. If the growth rate of these R- and S-type resistant mutants is decreased compared to the wild type, this could support using these toxins as a viable biocontrol option.

Chapter 2

Methods

Media Preparation

Two types of media were used in these procedures: King's Medium B (KB) and Luria-Bertani Medium (LB). *Pseudomonas* or mixed cultures were grown on KB while *E. coli* was grown on LB. KB medium was made by combining 10 g of proteose peptone #3 (BD, Franklin Lakes, NJ), 10 g of Tryptone (BD), 10 mL of 860 mM K_2HPO_4 (Sigma-Aldrich, St. Louis, MO) , 10 mL of 609mm $MgSO_4$ (Sigma-Aldrich), and 10.26 g of glycerol (Sigma-Aldrich) then adding MilliQ water to a total volume of 1 L. LB medium was produced by combining 10 g of tryptone (Fisher, Waltam, MA), 10 g of NaCl (Sigma-Aldrich), and 5 g of yeast extract (Fisher) and adding MilliQ water to a total volume of 1 L. These media were sterilized by autoclaving at 121^{o}C for 30 minutes. To produce agar media rather than liquid media, 15 g/L of agar (APExBIO, Houston, TX) was added before sterilization. Various antibiotics were required for this procedure with the working concentrations for those used shown in Table 1. Antibiotics were added to the media after autoclaving and cooling to a temperature of 55^{o}C to avoid degradation.

Table 1: Working Concentrations of Relevant Antibiotics

Antibiotic	**Abbreviation**	**Working Concentration (µg/mL)**
Gentamicin	Gent	25
Nitrofurantoin	NFT	50
Rifampicin	Rif	50
Kanamycin	Kana	50
Tetracycline	Tet	10
Mitomycin C	Mito	0.05

Bacteriocin Resistant Mutant Generation

Mutants of *P. syringae pv. actinidiae* BV3 resistant to both the R-type and S-type bacteriocins produced by *Psy* B728a were isolated in an attempt to identify the genetics underlying S-type bacteriocin sensitivity. Because of the conditional redundancy of these bacteriocins, this could only be done in a stepwise manner. A mutant of *Psy* B728a was created in which the S-type bacteriocin was deactivated (*Psy* B728a Δ4651) and its supernatant was harvested and sterilized by pelleting the cells and running through a 0.2 µm filter. *Psa* BV3 was grown overnight in KB medium. The optical density at 600 nm (OD 600) of the overnight culture was then measured and normalized to 0.1. The *Psa* BV3 culture was then diluted 1:10 into KB medium and incubated at 28^{o}C for 3-4 hours until reaching log phase.

In a 96 well plate, 10 µL of log phase *Psa* BV3 was added to 90 µL of sterile *Psy* B728a Δ4651 supernatant and was pipetted to mix. The plate was then allowed to incubate at room temperature for 30 minutes before all 100 µL was spread onto a KB plate. The plates were incubated at 28^{o}C for 48 hours until colonies arose. These colonies were then screened with subsequent exposure to *Psy* B728a Δ4651 supernatant to ensure they were true R-type resistant mutants rather than tolerant cells. This procedure selected for lipopolysaccharide (LPS) mutants of *Psa* BV3 which were resistant to the R-type bacteriocin.

An LPS mutant, R-type resistant strain provided by another lab, designated A2, was grown overnight, normalized, and allowed to reach log phase via the same procedure as above. In a 96 well plate, 10 µL of log phase A2 was added to 90 µL of sterile wild type *Psy* B728a supernatant before being incubated and plated as described above. The colonies were then screened with *Psy* B728a supernatant to ensure the colonies were resistant. This procedure selected for mutants of *Psa* BV3 resistant to both the R-type and S-type bacteriocins produced by *Psy* B728a. Twelve R-

type and S-type resistant *Psa* BV3 strains were generated in this manner, which share the same mutation conferring R-type resistance but have independently derived S-type resistance.

Spotting Procedure of Resistance Screening

A broth culture of the strain of the strain of interest was grown in KB medium overnight at 28ºC. This culture was then diluted 1:1000 in KB medium and allowed to incubate for a further 3-4 hours to ensure the cells were in log phase. Two 5 µL spots of the log phase culture were then placed on a KB plate. The same supernatant used in the bacteriocin resistant mutant generation procedure was then pipetted in the amount of 8 µL onto one of the spots. The spots were allowed to dry on the plate before it was placed in a 28ºC incubator for 24 to 48 hours. By this point, the untreated spot should have grown into a solid lawn of bacteria where the spot was applied and serve as a comparison. If the strain is resistant to the bacteriocin, the treated spot will look the same, just slightly larger than the untreated spot. If the strain is not resistant, the treated spot will have few to no colonies present.

Mutant Genomic DNA Extraction, Sequencing, and Alignment

A 3 mL culture of each mutant strain was grown overnight in KB medium at 28ºC. This culture was then placed into individual 2-mL microcentrifuge tubes and the cells were pelleted. The supernatant was then discarded without disturbing the pellet. The cells were then lysed and the DNA purified using the Promega (Madison, WI) Wizard Genomic DNA Purification protocol for Gram-negative bacteria. The DNA in this procedure was rehydrated in water. The DNA concentration, 260/280, and 260/230 values were then recorded using a Nanodrop XTYPEX

machine. The DNA was then sent for Illumina whole genome sequencing at the Penn State Genomics Core Facility (University Park, PA). The sequencing data was uploaded onto the Geneious software and aligned using the genome of the LPS mutant parent strain, A2. This strategy was used to identify the genes and mutations present in mutant strains compared to the wild type.

Primer Design

For selected mutant strains (P1B1, P2D1, P2E1, and P6A1, described in Table 3), forward and reverse primers were designed to amplify the genomic region around a mutation observed in the Geneious software. The resulting segments amplified by these primers were around 1700 base pairs in length. Additionally, primers were designed with the binding region overlapping with the observed mutation to serve as a molecular check to distinguish the mutant and wild type segments.

Gateway Cloning

In order to test whether the observed mutations in the R-type and S-type resistant *Psa* BV3 strains, were causative of the resistance pattern observed, gateway cloning was used to swap the segment amplified by the designed primers between the mutant with that of the wild type. This process is visually outlined in Figure 4. To do so, genomic DNA was extracted from the A2 LPS mutant parent strain (referred to as wild type) as well as the P1B1, P2D1, P2E1, and P6A1 strains of R-type and S-type resistant mutants (referred to as mutants). The segments surrounding the mutations were then amplified in mutant strains using Phusion PCR and the outer primers designed to extract a region of interest about 1700 base pairs in length. The same regions were also amplified in the wild type strain. This yielded 4 pairs of wild type and mutant genomic regions.

The PCR products were then cleaned and a subsequent Phusion PCR was run to add att-B tails to the flanks of each region. The flanked regions were then inserted into the pDONR 207 plasmid via the BP Clonase reaction. The pDONR 207 plasmid is a vector containing a gentamicin resistance gene. The BP Clonase product was then used to transform *E. coli* TOP10 cells through a heat shock procedure. The transformed cells were plated in two unequal volumes on LB plates with gentamicin for selection. The plates were then incubated overnight at 37ºC until colonies appeared. Colonies were screened through a *Taq* colony PCR using the outer primers to ensure the region could be successfully amplified out of *E. coli*. Successfully transformed colonies where then grown overnight at 37ºC in 3 mL of LB medium with gentamicin. Equal parts of this culture and 80% glycerol (usually 500 µL each) were then mixed to create a glycerol stock and stored at -80ºC. The plasmid was harvested from two mL of the overnight culture using a QIAGEN (Germantown, MD) plasmid mini prep procedure. The purified plasmid was then quantified using a nanodrop machine.

The purified pDONR 207 plasmid containing the gene segment was then inserted into a suicide vector, pMTN 1907, through the LR Clonase reaction. The pMTN 1907 vector contains a killing gene at the insertion site activated in the presence of sucrose as well as a tetracycline resistance gene. The LR Clonase product was then used to transform *E. coli* TOP10 cells through the heat shock procedure. The transformed cells were plated in two unequal volumes on LB plates with tetracycline for selection. The plates were then incubated overnight at 37ºC until colonies arose. Colonies were then screened through a *Taq* colony PCR using the outer primers to ensure the region could be successfully amplified out of *E. coli*. Successfully transformed colonies were then grown overnight at 37ºC in 3 mL of LB medium with tetracycline. Equal parts of this culture and 80% glycerol (usually 500 µL each) were then mixed to create a glycerol stock and stored at

-80°C. The plasmid was harvested from two mL of the overnight culture using a plasmid mini prep procedure. The purified plasmid was then quantified using a nanodrop machine.

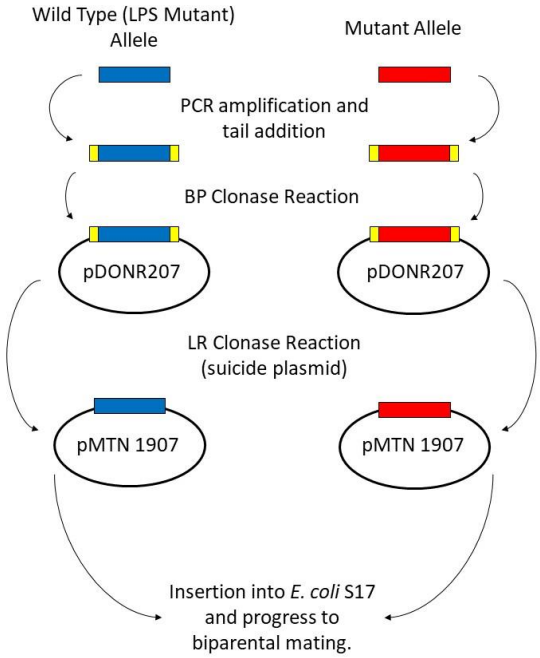

Figure 4: Diagram of Gateway Clonase Process

Heat Shock Transformation Procedure

The heat shock transformation procedure was used to insert plasmids into *E. coli* strains during the Gateway cloning procedure and in preparation for the biparental mating procedure. A 100 µL vial of chemically competent *E. coli* TOP10 or *E. coli* S17 cells was thawed on ice. Between 1 and 5 µL of the plasmid to be inserted was pipetted into a 2-mL microcentrifuge tube. The TOP10 cells were then pipetted into the tube and gently mixed by stirring before being allowed to incubate on ice for 30 minutes. The tube was then heat shocked for 30 seconds in a 42°C water bath before being placed back on ice for 2 minutes. S.O.C. medium was added to the tube in the

amount of 250 µL. The tube was then placed horizontally and incubated at 37ºC and 225 rpm for 1 hour. The cells were then plated at a range of dilutions on LB plates with the appropriate antibiotic for selection. The plates were incubated at 37ºC overnight until colonies arose. Colonies were screened for the appropriate genomic region using *Taq* Colony PCR. Positive colonies were grown overnight in LB medium with antibiotic if appropriate at 37ºC. Equal parts of this culture and 80% glycerol were then mixed to create a glycerol stock and stored at -80ºC.

Biparental Mating

E. coli S17 cells containing the pMTN 1907 plasmid from the Gateway cloning procedure with the gene section inserted (donor strain) were grown overnight at 37ºC. Other than the donor strain overnight, all incubations took place at 28ºC. The *Pseudomonas* strain into which the segment was to be inserted, either A2 or R- and S-type resistant mutant (recipient strain), was grown overnight in KB medium. In this experiment, each genomic region of interest was to be mated into the opposite genetic background. The donor and recipient cultures were then pipetted into separate microcentrifuge tubes and spun at maximum speed for 1 minute to pellet the cells. The supernatant was discarded. The culture was then resuspended in 1 mL of KB medium before being pelleted again and decanting. This procedure was repeated for a total of two washes. The pellet remaining after the final wash was resuspended in 200 µL of KB. Equal parts of the donor and recipient strains (100 µL each) were then combined in a new microcentrifuge tube and were thoroughly mixed by pipetting or vortexing. Spots of the mixed culture 50 µL in volume were placed on KB plates and allowed to dry. The plates were then incubated for 72 hours to create 3-4 large bacterial masses where spots were applied.

To remove the bacterial masses, 1 mL of 10 mM $MgCl_2$ was applied to the plate and a hockey stick was used to scrape the surface of the plate. The plate was tilted and the liquid was pipetted out into a microcentrifuge tube. This process was repeated to rinse the plate and recover as many cells as possible. Aliquots of 100 µL of the recovered cell solution were plated onto each of 10 KB plates with tetracycline, NFT, and rifampicin that were prepared in the previous 48 hours. These plates were then incubated for 48 to 72 hours until colonies arose. These colonies were patched onto another KB plate with the same antibiotics to ensure resistance. The plates were incubated for another 48 to 72 hours until single colonies were visible. These colonies were then picked and resuspended in 50 µL of molecular grade water. A test tube containing 3 mL of KB medium with tetracycline and was then inoculated with 20 µL of the resuspension and incubated overnight.

The overnight culture was then used to inoculate KB at a 1:25 ratio and was grown for 4-6 hours to reach log phase. It was then diluted to 10^{-4}, 10^{-5}, and 10^{-6} in KB medium. Aliquots of 100 µL of each diluted log phase culture was plated onto each of two KB plates amended with 5% Sucrose, which was dissolved in the medium after autoclaving. This process was used to remove the pMTN 1907 plasmid from the recombinant strain, as the killing gene is activated in the presence of sucrose. These plates were then incubated overnight. Single colonies were resuspended in 50 µL of water. Overnight cultures in KB and KB with tetracycline in a 96 well plate were then made, using 10 µL of the resuspension as inoculant for each well. Any cultures that grew in the culture with tetracycline were discarded, as this meant the plasmid backbone was integrated into the genome. A PCR was performed on the tetracycline sensitive strains using the primers located directly on the mutation site to determine whether the gene incorporated was the wild type or mutant version. Glycerol stocks were made of confirmed recombinants.

Harvesting Supernatant

In order to investigate resistance to bacteriocins and effects of metabolites without using mixed cultures, sterile supernatants were generated. To do so, a flask containing the desired volume of KB medium was inoculated with the desired *Pseudomonas* strain and allowed to grow overnight in a 28ºC incubator shaking at 215-225 rpm. After growing overnight, the OD 600 of the culture was recorded before placing the culture in conical tubes. The tubes were spun for 10 minutes at 4500 rpm (2000g) to pellet the cells. The supernatant was then transferred into sterile syringes and run through a 0.2 micron filter into a fresh conical tube. For volumes too large for syringe filtration, cultures were run through 0.2 micron filters in a vacuum filtration unit. Sterile cultures were stored at 4ºC until use. This procedure was used to generate supernatant from *Psa* BV3, *Psy* B728a, *Psy* B728a Δ4651 (R-type bacteriocin deactivated), and *Psy* B728a ΔRΔS (both the R-type and S-type bacteriocin deactivated). To harvest supernatant with higher levels of bacteriocin present for use in resistance screenings, the culture had to be induced. To do so, an overnight culture of the desired strain was added 1:1000 to sterile KB medium and grown for 4-6 hours until it reached log phase. Mitomycin c was then added to a concentration of 0.05 µg/mL and the culture was allowed to incubate overnight. The overnight culture was then processed in the same manner as above for the regular spent supernatants.

Growth Assays

Anecdotal observations of mutant strains suggested that there was a difference in growth rate between some mutant and wild type strains. In order to determine whether these observations were true and significant, a growth assay was performed. The strains of interest, including BV3

(wild type, parent strain of A2), A2 (LPS mutant and parent strain of all subsequent mutants), P2D1 (regular growth rate mutant), P6A1 (regular growth rate mutant), P1B1 (slow growth rate mutant), and P4G2 (slow growth rate mutant) were streaked out onto KB plates. After single colonies were observed, they were used to inoculate cultures of KB medium and were grown overnight. All incubations during the growth assay took place at 28ºC. The overnight cultures were then normalized to an optical density at 600 nm (OD 600) of 1.0. The normalized cultures were added in the amount of 300 µL to test tubes containing 2.7 mL of KB medium to reach a starting OD 600 of approximately 0.1 and starting volume of 3 mL. This procedure was completed in triplicate for each strain. The OD 600 of the cultures was then measured during growth at 0, 1, 3, 4, and 5 hours.

Because all mutants arose at the same time during generation, it was believed that the growth rate of the apparent slow growers could be recovered by the metabolites in either the *Psy* B728a or *Psa* BV3 supernatants. The strains selected for this assay were P1B1, a slow grower, and A2, its LPS mutant parent strain. The same procedure as the first trial was used, except the normalized cultures were added in triplicate to tubes containing 2.7 mL of *Psa* BV3 supernatant, B728a ΔRΔS (R-type and S-type bacteriocins deactivated) supernatant, and KB medium. B728a ΔRΔS supernatant was used to allow for growth of the A2 strain, which is susceptible to the S-type bacteriocin. OD 600 measurements were taken at hours 0, 1, 3, 4, and 5.

During data analysis, the recorded OD values at 600 nm were natural log transformed and plotted against time. The slope of the best fit line of the 3, 4, and 5-hour ODs was determined, which corresponds with the average growth rate over that time period. The average growth rate was then converted to doubling time by dividing the ln(2) by the growth rate. Statistical significance of the differences was determined using a one factor ANOVA with a p value of 0.05

selected as the level of significance. Percentage difference between doubling times was calculated by subtracting the doubling time of the treatment strain from the KB medium control doubling time and dividing by the KB medium control doubling time, then multiplying the result by 100.

Chapter 3

Results and Discussion

Resistant Mutant Genetic Analysis

Region of Common Deletion within Mutant Strains

The 12 R- and S-type resistant mutants that were generated had their genomic DNA extracted and were whole genome sequenced by Illumina sequencing. The resultant DNA fragments were then mapped to the genome of the LPS mutant, R-type resistant parent strain, designated A2, using the Geneious software. These alignments revealed that 9 of the 12 mutants had sections of around 50 genes deleted in a common genomic region as shown in Figure 5.

The commonly deleted genes spanned a length of about 102000 base pairs (bp) compared to the genome length of about 6.5 million bp, about 1.5% of the genome. The mutants with deletions can be divided into two groups based on the approximate start and stop locations of their deletions, left skewed and right skewed. The left skewed P1A1, P2G2, and P4D1 strains had deletions running from the left flank of the glycoside hydrolase family protein to a right flank of glutathione S-transferase gene. The right skewed P2D1, P2E1, P2G1, P4E1, P4G2, and P4K1 strains, however, had a left flank of the N-acetyltransferase gene and a right flank of the methyl-accepting chemotaxis protein. Both groups shared the loss of a central region of genes spanning

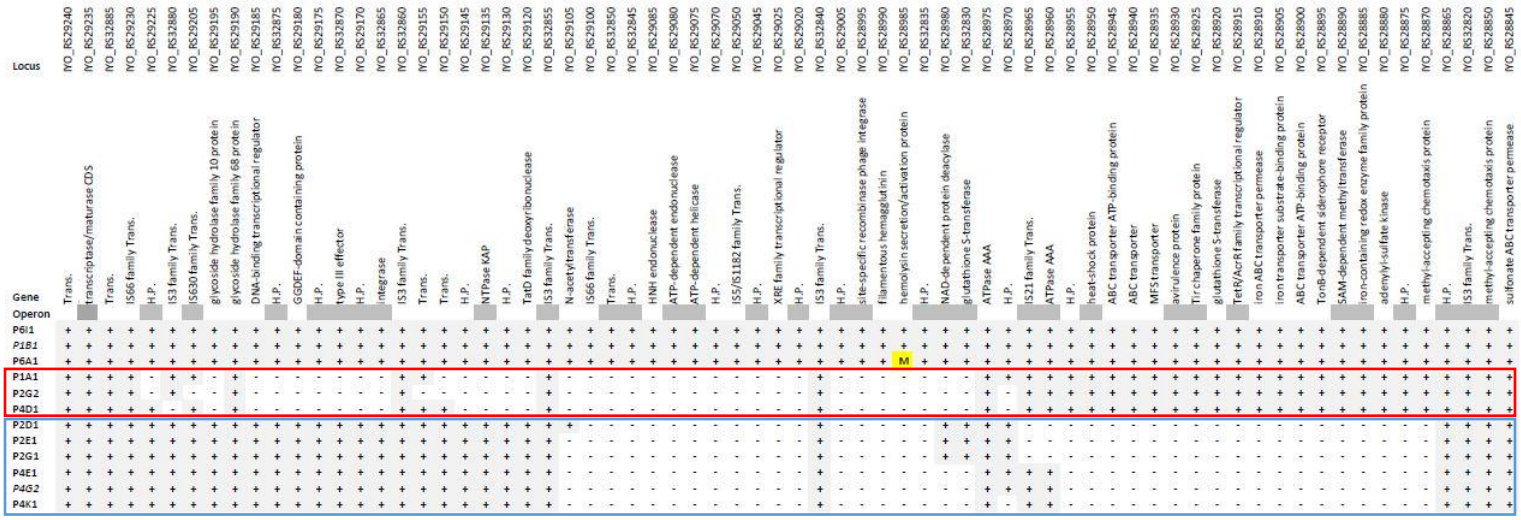

Figure 5: Gene Deletion Regions in R- and S-type Resistant Mutants

(This figure displays the presence (grey box with +), absence (white box with -), and mutations (yellow box with M) in the genomic region of common deletion within the R- and S-type resistant mutants. The mutant strain designations are listed on the left side, with the italicized strains (P1B1 and P4G2) displaying a slower growth rate. Suspected operons are grouped by the white or dark grey boxes located under the gene names. In the gene names, hypothetical proteins are designated H.P. while transposons are designated Trans. The locus tag of the gene within the genome is listed in the top row of the figure. P6I1 and P1B1 have mutations in the *hflB* and PQQ-dependent catabolism-associated beta propeller genes respectively elsewhere in the genome. The strains contained within the red box are considered 'left skewed' in their deletion while those in the blue box are 'right skewed' in their deletion.)

from the N-acetyltransferase to the hemolysin secretion/activation protein. There were no strains that had a deletion extending the full length of the deletion region (from the glycoside hydrolase gene to the methyl-accepting chemotaxis protein). There could be two reasons for this. The first is that loss of the flanks on both sides of the central region is fatal or results in so slow a growth rate that these mutants were not recovered. Alternatively, the two variations on the deletion region could simply be the most biochemically or recombinationally favorable ways for the region containing the gene important for the development of resistance to loop out of the genome.

Another mutant without a region of deleted genes, designated P6A1, which had a single base pair deletion leading to a frameshift mutation within the hemolysin secretion/activation gene in the central deletion region. This suggested that loss or deactivation of this gene was important for developing resistance to the S-type bacteriocin.

Another gene of interest within this region was the TonB dependent siderophore receptor, which was lost by the right skewed mutants but retained by the left skewed mutants. This receptor has been shown to be a bacteriocin target in *E. coli* and *Pseudomonas aueriginosa*, which suggested it could be targeted in this system (Lazdunski et al. 1998, Ghequire & Ozturk 2018).

In addition to the P6A1 strain, there were two other strains that did not have any regions of deletion (P6I1 and P1B1). These strains did, however, have a mutated gene elsewhere in the genome. While P6I1 had a single base pair insertion causing a frame shift in the *hfl*B gene, P1B1 had a single base pair insertion 20 bp upstream of the PQQ-dependent catabolism-associated beta propeller gene. The mutations in or near these genes in R- and S-type resistant mutants suggested that these genes could also be important for development of S-type bacteriocin resistance.

Some genes, like the IS3 family transposase gene in the central deletion region appeared to be retained by all strains. The could be artifactual, as Illumina sequencing works by cutting the genome into short fragments that are then sequenced and recorded. These fragments can then be mapped to a genome using software like Geneious. This means that fragments from genes with multiple copies in the genome can appear to be mapped into a region that they are not actually present. This phenomenon had to be considered when transposases or other genes appeared to be the only gene retained in a region that had otherwise been lost.

Identity and Function of Genes Affecting the Target of the S-type Bacteriocin

From the examination of the aligned mutant genomes, four potentially important genes for the development of S-type resistance were identified, as summarized in Table 2. One of the most compelling of these genes was the hemolysin secretion/activation protein, which was altered or absent in 10 of the 12 mutants analyzed in this project. This gene was contained in the central region of deletion common to both the right and left skewed groups, meaning it was lost completely in 9 of the mutants. The other mutant, P6A1, did not have the gene fully deleted, but experienced a single base pair deletion. The deletion of this guanine caused a frameshift within the gene and a premature stop codon, cutting off 1452 base pairs of the total 1680 base pair length. A mutation with this degree of disruption to the amino acid chain is likely to result in a protein unable to perform its normal function.

Hemolysin secretion/activation proteins are most extensively studied in the system of *Serratia marcescens,* a blood pathogen, where these proteins release and activate hemolysin, which can cause lysing of red blood cells (Pramanik et al. 2014). The lysing of the blood cells frees the heme, which *S. marcescens* then uptakes. The hemolysin lyses cells by creating pores in the phospholipid bilayer, eventually causing cell death (Chalmeau et al. 2011). A similar mechanism for lysing competing bacteria, rather than blood cells could be effective in increasing the amount of free iron present in the environment. Bacteriocin targeting of this gene could therefore impose a fitness cost in iron-limited environments for mutants resistant to the S-type bacteriocin.

Iron has been shown to be a prominent growth-limiting factor in the growth of *P. syringae* pv. *tomato* on minimal media (Kim et al. 2009). One way to increase uptake of iron is to release pyoverdine, a siderophore, which chelates iron in the environment (Taguchi et al. 2010). TonB dependent uptake receptors then recognize the pyoverdine and transport the chelated iron into the

cell. The importance of iron uptake through this mechanism is underscored by many *Pseudomonas* strains encoding multiple TonB receptors recognizing not only their own siderophores, but those produced by competing strains (Hartney et al. 2013). These receptors have also been shown to be the target of pyocins in *P. aeruginosa*, which produces a toxin targeting its own receptor to discourage competitors from retaining the ability to uptake its siderophores while avoiding killing itself by producing an immunity protein simultaneously (Ghequire & Ozturk 2018). A similar system could be employed by *Psy* B728a to prevent *Psa* BV3 from using its siderophores. The loss of this gene in over half of the recovered mutants suggested that loss of this receptor may play a role in developing resistance to the S-type bacteriocin.

The P6I1 non-deletion strain contained a mutation in the *hflB* gene outside of the commonly deleted region. This was a single base pair insertion which led to a frameshift mutation and a premature stop codon 334 bp before the end of the gene, which is a total of 1906 bp in length. While much of the gene was still intact, this mutation likely decreased the efficiency of the *hflB* gene. This decreased function is especially important in bacteria, which usually retain only a single copy of the gene, which was the case for the strains of *P. syringae* used in this study. This gene encodes ATP and Zn dependent proteases, which are involved with functions like assembly of membrane proteins and protein secretion (Seemuller et al. 2011). While these proteases likely could not be directly recognized by a bacteriocin, inactivating this gene could prevent the successful assembly of the membrane protein or receptor that the S-type bacteriocin recognizes.

The P1B1 non-deletion strain contained a single base pair insertion 20 bp upstream of the start of the PQQ-dependent catabolism-associated beta propeller gene. While this insertion was not in the gene itself, it was in a section of repeating base pairs that could be a promoter. Decreasing the efficiency of the promoter could then functionally inactivate the downstream gene. The PQQ-

dependent catabolism-associated beta propeller gene commonly occurs as part an operon responsible for the dehydrogenation of alcohols (Matsumura et al. 2014). The enzymes in this process are often located in the periplasm or the membranes, meaning that a loss in function of this operon could lead to lack of production of the enzyme recognized by the S-type bacteriocin (Umezawa et al. 2015).

Identification of these genes of interest as candidates for S-type bacteriocin targeting through alignment with the parent strain suggested they could play a role in the development of resistance. Examination of the functions of these genes further reinforced this hypothesis, as all were associated with import or export receptors which could be recognized by S-type bacteriocins.

Table 2: Potential Genes Affecting the Target of the S-type Bacteriocin

Gene	**Number of Strains with Alteration**	**Mutation Type**	**Potential Function**
hemolysin secretion/activation protein	10 of 12	full gene loss (deletion mutants) or 1 bp deletion frameshift (P6A1	Associated with release of compounds encouraging iron uptake
TonB dependent siderophore receptor	7 of 12	full gene loss (right skewed mutants)	Uptake of iron-containing compounds like heme or pyoverdine
hflB	1 of 12	1 bp insertion frameshift (P6I1)	Associated with protein secretion and membrane protein assembly
PQQ-dependent catabolism-associated beta propeller	1 of 12	1 bp insertion 20 bp upstream of gene (P1B1)	Catalyst for oxidation of alcohols and aldose sugars in periplasmic space

Concept and Selection of Strains for Gateway Cloning and Biparental Mating

While the genetic identification of mutations and gene losses suggests these genes play a role in developing S-type bacteriocin resistance, it does not prove this hypothesis. In order to confirm the mutations as the cause of S-type bacteriocin resistance, the altered gene must be cloned into the parent background and vice versa to observe whether the resistance pattern changes consistently with expectations as shown in Figure 6.

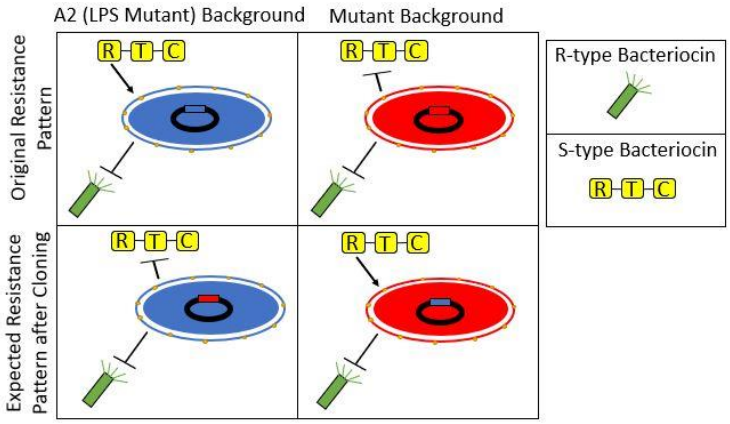

Figure 6: Confirmation of Mutation as Cause of S-type Bacteriocin Resistance

Four strains, outlined in Table 3, were selected were selected to undergo this cloning process. Because there is a limit on the size of section that can be successfully cloned into the opposite background, strains were selected that had mutations in genes that were present rather than genes that were deleted. Two of these strains contained the region of deleted genes in addition to a mutation in another gene while the two others had no deletion region.

Table 3: Strains Selected for Gateway Cloning

Strain	Altered Gene	Mutation Description	Region of Deleted Genes
P1B1	PQQ-dependent catabolism-associated beta propeller gene	single bp insertion 20 bp upstream of the start of gene	No
P2D1	methyl-accepting chemotaxis protein	C to A nucleotide transversion leading to a premature stop codon	Yes
P2E1	12-oxophytodienoate reductase	G to T nucleotide transversion 2 bp upstream of start of gene	Yes
P6A1	hemolysin secretion/activation protein	single bp deletion leading to frameshift/premature stop codon	No

The selection of strains with mutated, but present genes allowed for greater control over the variables that could cause differences in resistance. While the swap of a single mutated gene between the mutant and wild type strain causing a change in resistance pattern confirms that gene's role in the change, the same swap with a section of deleted genes would only determine the region of genes responsible.

For each of these four strains, primers were designed to amplify an approximately 1500 bp region surrounding the mutation in the mutant strain. The same primers were used to amplify the same section without the mutation. This created a total of 8 constructs to be progressed through the gateway cloning procedure. Additionally, diagnostic primers were designed to rest directly on top of the observed mutation. This would allow amplification of a segment when the mutation was present, but not when the mutation was absent and would serve as a diagnostic PCR. Once each construct completed the gateway cloning procedure and the appropriate genotype was confirmed via the diagnostic PCR, the section was to be inserted into the opposite genetic background (mutant into wild type, wild type into mutant) through biparental mating. Seven of the 8 constructs had

completed the gateway cloning procedure, but had not been successfully mated into the opposite genetic background.

Progress and Challenges in Gateway Cloning and Biparental Mating

As this was the first time Gateway cloning had been used in this lab, optimization of the procedure was important for ensuring its success. Despite repeated attempts of optimization, the cloning of the wild type version of the hemolysin secretion/activation protein was unable to be completed. Repeated attempts to move this segment into the pDONR207 vector in the BP Clonase reaction were unsuccessful. This could be due to an issue in the interaction of the attB tails necessary for insertion of the segment.

While biparental mating was attempted with the 7 strains that had completed the gateway cloning procedure, successful completion of this procedure was not achieved. While resultant colonies from this process were supposed to behave like the parent colonies, the resultants from this procedure had different behavior and morphology from either the mutant or wild type strains as shown in Figure 7. The colonies still fluoresced under UV light as is expected from these strains, which are florescent pseudomonads, however it usually took a few days to a week before this is visible. The resultant colonies displayed this phenotype immediately upon becoming visible. Additionally, these resultant colonies grew more quickly than expected, arising after one day instead of two as was expected.

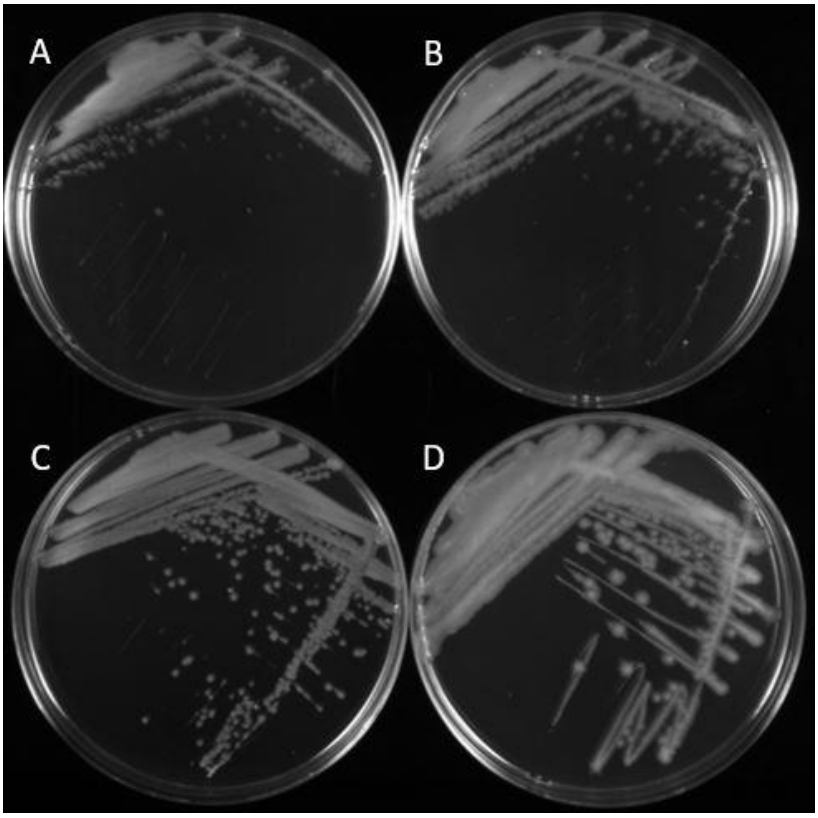

Figure 7: Biparental Mating Resultant Strain compared to Parent Strains

(After a 48-hour incubation, the resultant strain from the biparental mating of the P2D1 genetic segment into the A2 background yields colonies (D) that do not resemble those of the A2 (A) or P2D1 (B) parent strains or the *E. coli* S17 (C) used as a vector for the plasmid in this protocol.)

There are two possible explanations for why this occurred. The first is that the phenotype is a response to the stresses of the biparental mating procedure, during which cocktails of antibiotics are used for selection and there are briefly two copies of the gene region being cloned into the opposite background. Another explanation is that these resultants are another strain or species of *Pseudomonas*. This could occur if there is contamination during the biparental mating procedure. Because the resultant mutant does not appear to have a colony morphology or growth

rate like that of either the parent *Pseudomonas* strains or the *E. coli* used to introduce the pMTN 1907 vector, it is most likely that contamination is occurring during this procedure.

An additional challenge was the difficulty in optimizing the diagnostic PCR. Because the diagnostic primers were designed to lay directly on top of the mutation, it left no choice for the sequence that had to be used as the primer. This meant that the strength of the primer was not ideal. The conditions necessary to allow for use of these primers were not found during this project, meaning that mutant and wild type strains could not be distinguished during the cloning or mating procedures except by sequencing.

Growth Rate Analysis

Challenges and Limitations in the Completion of Growth Rate Assays

While the concept of a growth assay is simple, there were a few factors that complicated this undertaking in the project. One of the most notable was a clumping phenotype displayed by the A2 (LPS mutant) as well as all of the R- and S-type resistant mutants as shown in Figure 8.

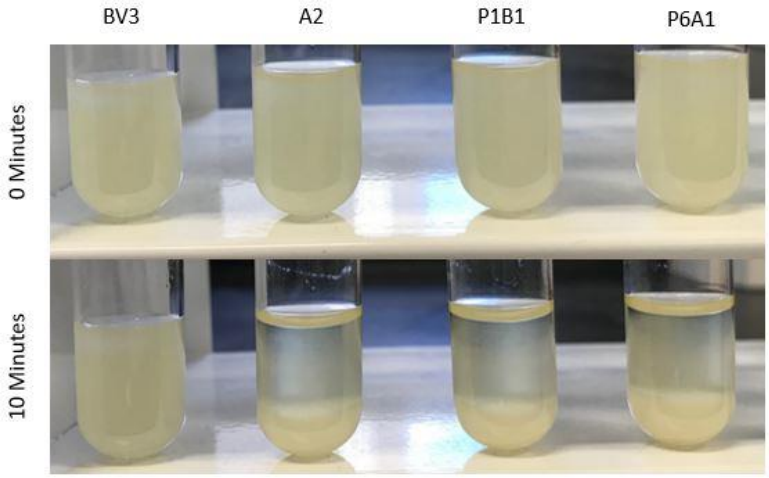

Figure 8: Settling of LPS mutants compared to BV3

(Settling of LPS mutants (A2, P1B1, and P6A1) occurs rapidly following conclusion of shaking compared to the wild type (BV3). Significant settling occurs even in the first 10 minutes for this phenotype, which includes A2 and the 12 R- and S-type resistant mutants generated.)

While the mixing in test tubes was sufficient to keep the cells in suspension, it was not sufficient in smaller receptacles. Specifically, 96 well plates would have been preferred for completing this experiment as they would have allowed more replicates and treatments, the use of

an electronic plate reader for OD measurements, and use of less materials. Due to this constraint, the scope of the growth assay had to be reduced from initial intentions.

Additionally, working with strains having markedly different growth rates proved difficult. While normalizing OD was straightforward, it was more difficult to ensure that the strains were at the same stage within their growth curve. After incubating overnight, cultures of normal growth rate strains tended to be in the stationary phase, while those of slow growing cultures were still in log phase. This resulted in the slow growing strain initially outgrowing the normal growth rate strains for the first 1 to 2 hours as they experienced a lag. After this point, the normal growth rate strains had adjusted to the environment and entered log phase, where its growth rate separated from that of the slower growing strains. Because of this, the doubling times in this project were calculated using only the OD from hours 3, 4, and 5 of the assays.

The differences in growth rate were also an issue when generating cultures to turn into supernatants. While ideally the OD of these cultures at the time of harvest would be as similar as possible, it was difficult to ensure consistent culture density. Because of this, not only the strain from which the supernatant was harvested had to be considered, but how depleted the nutrients in the culture were likely to be compared to the others.

Selection of Strains for Use in Growth Rate Assays

Anecdotal observation of the mutant strains suggested that some of the strains grew more slowly than others. When streaking the strains from glycerol stocks onto KB plates, *Pseudomonas* usually took two days for colonies to grow large enough to see and use. The P1B1 and P4G2 strains, however, took three days to reach this point. This difference is shown in Figure 9.

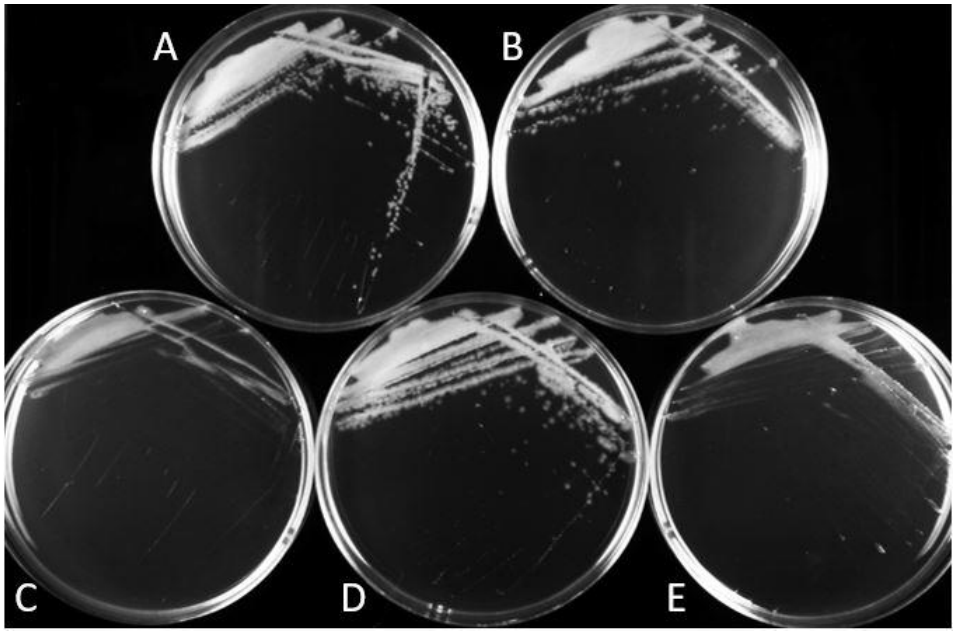

Figure 9: Difference of BV3 and Mutant Colony Size after 48 hours Incubation

(While single colonies are large enough to be visible in the BV3 (A), A2 (B), and P2D1 (C) strains, no single colonies are yet visible for P1B1 (C) or P4G2 (E).)

This led to the decision to test whether this growth rate difference was significant in a liquid culture assay. In selecting the strains for the assay, genetic background was taken into account. The slow growing strains already had a mutant with (P4G2) and without (P1B1) the region of deleted genes represented. The P2D1 and P6A1 strains were selected because they also did and did not have deletion regions respectively. The BV3 (wild type) and A2 (LPS mutant parent strain) were also included as controls. This assay could then inform whether growth rate had decreased and whether this decrease was linked to regions of deletion. The selected strains are summarized in Table 4.

Table 4: Selected Strains for Growth Rate Assay

Strain	Region of Deleted Genes	Expected Growth Rate	Description
BV3	No	Normal	Wild Type Strain
A2	No	Normal	LPS Mutant, Parent Strain
P2D1	Yes	Normal	R- and S-type resistant mutant
P4G2	Yes	Slow	R- and S-type resistant mutant
P6A1	No	Normal	R- and S-type resistant mutant
P1B1	No	Slow	R- and S-type resistant mutant

For the subsequent assay involving growth in varying supernatants, P1B1 was selected as the slow growing strain for two reasons. The first was that its growth rate was found to be slower than the of P4G2 by the initial assay, meaning that the growth rate recovery could be easier to recognize. Additionally, the lack of deletion region in the P1B1 strain allows for more targeted inquiry when seeking to explain the observed phenotypes, since only a single mutation separated this strain from its A2 parent. The genetic similarity was the reason that A2 was selected as the control strain rather than BV3.

Growth Rates of Selected Strains

The six strains selected for the reasons stated above were grown in liquid KB media and the OD was measured at 1, 3 ,4 and 5 hours after inoculation with normalized amounts of overnight culture from each strain. The doubling times for these strains were calculated using the OD

measurements from hours 3, 4 and 5 of the assay. The average doubling time for each strain is shown in Figure 10.

As would be expected from anecdotal observation, P1B1 and P4G2 had statistically slower growth rates than the other tested strains at 4.75 and 3.44 hours respectively. The growth rates of these strains were also statistically different from each other. This suggested that the slow growing phenotype is a spectrum or tiered phenotype rather than a binary slow or normal. This is likely due to the different mechanisms by which the S-type resistance can be developed.

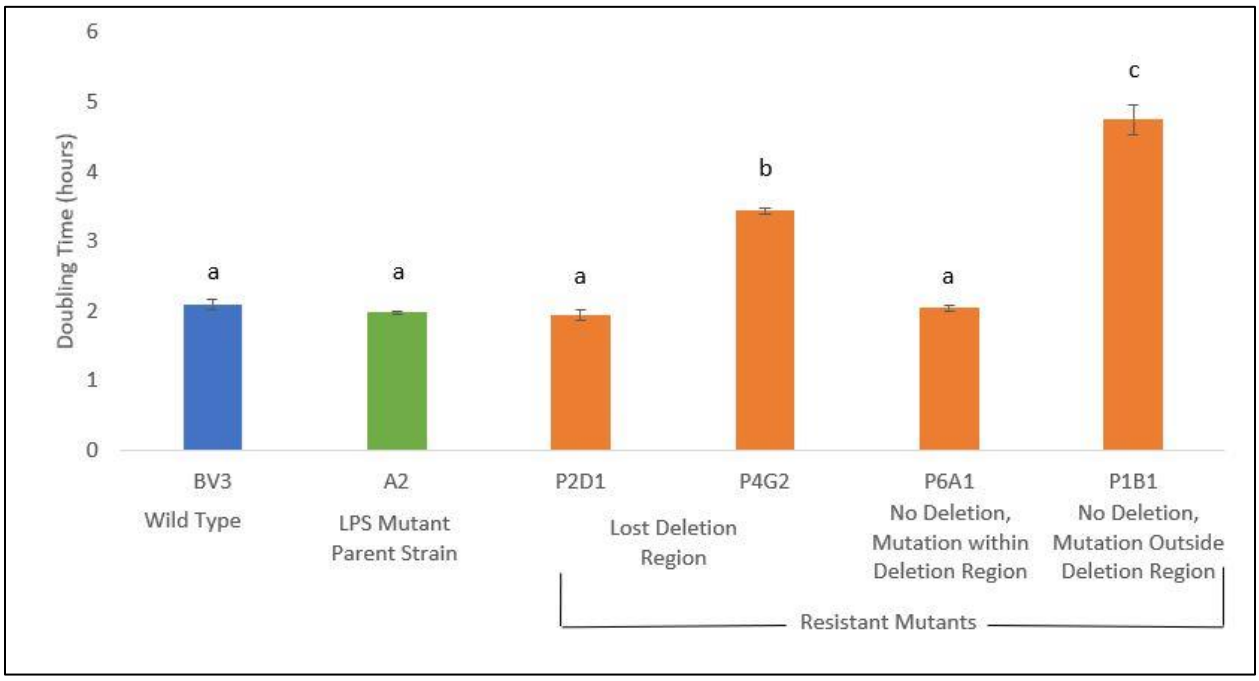

Figure 10: Growth Rates of Selected Strains

(Growth rates of selected strains as shown by doubling time. BV3 (blue) is the wild type strain while A2 (green) is an LPS mutant and the parent strain of the other mutants (orange) which are labeled with descriptions of their genotypes. Error bars are ±1 SD. Groups a, b, and c are statistically different from one another ($p<0.05$).)

The P6A1 and P2D1 mutants, grew at statistically the same rate as the wild type (BV3) and parent (A2) strain, which had doubling times of about 2 hours. This does not appear to be consistent with the classical understanding of bacterial competition, in which development of resistance to a toxin would incur a fitness cost. There are, however, more elements to fitness than growth rate. While these decreases in fitness may not be apparent in laboratory media, where there is no competition, ample nutrients, and conditions favorable for growth, it could manifest if the strain were growing naturally in the environment.

Additionally, this assay suggested that growth rate is not linked to the presence or absence of genes in the deletion region. While it could be expected that mutants that have lost more than 1% of their genome would experience a growth rate decline regardless of the genes affected, the presence of slow and normal growers both with and without the region of deleted genes showed that the relationship is not this simple.

Effect of Supernatants on Growth Rates

During the generation of mutants, all colonies seemed to appear at the same time. The slow growing phenotype only appeared after the colonies were streaked out onto fresh KB plates for isolation. This suggested that there is a compound produced either by the wild type strain or the bacteriocin producing *Psy* B728a strain that is responsible for growth rate recovery. To test this hypothesis, the P1B1 (slow growing) and A2 (normal growth) strains were grown in supernatant harvested from *Psa* BV3 and a *Psy* B728a ΔRΔS (both the R- and S-type bacteriocin deactivated), which was selected over the wild type B728a to avoid killing A2. The average doubling times for each strain and supernatant are shown in Figure 11.

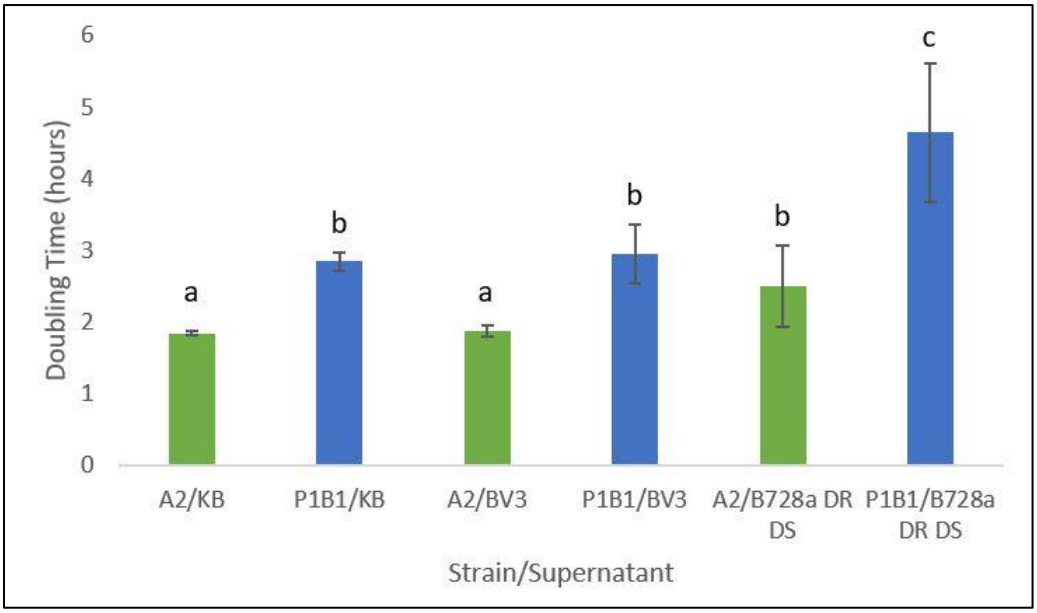

Figure 11: Growth of P1B1 and A2 in Varying Supernatants

(Doubling time from P1B1 (blue) and A2 (green) strains grown in KB medium, BV3 (wild type) supernatant harvested at an OD of 0.84, and B728a DR DS (R- and S-type bacteriocin deactivated) supernatant at an OD of 2.11. Error bars are ±1 SD. Groups a, b, and c are statistically different from one another ($p<0.05$).)

This assay suggested that these supernatants do not recover the growth rate of P1B1, which had statistically significant differences in doubling time compared to the A2 strain in all supernatants. The effect of the nutrient depletion in the B728a ΔRΔS supernatant, which had the highest OD at the time of harvest, was visible in that both the A2 and the P1B1 strains grew most slowly in this supernatant. The P1B1 growth rate appeared to have dropped more significantly than that of the A2 when compared to the KB growth rate. This is shown in Table 5 by the 63% increase in doubling time when grown in B728a ΔRΔS supernatant for P1B1 compared to only a 36% increase in doubling time for A2. This could be due to decreased fitness in nutrient stressed environments caused by the same mutation leading to its S-type bacteriocin resistance.

Table 5: Differences in Doubling Time Compared to that in KB

Strain/Supernatant	Doubling Time (hours)	% Difference compared to A2 Doubling Time
A2/KB	1.84	-
A2/BV3	1.87	1.76%
A2/B728a ΔRΔS	2.49	35.54%
P1B1/KB	2.84	-
P1B1/BV3	2.94	3.70%
P1B1/B728a ΔRΔS	4.64	63.25%

Chapter 4

Conclusions and Future Research

Psa BV3 caused severe economic damage in the New Zealand kiwifruit industry following its introduction in 2010. The competition between *Pseudomonas* strains could allow for the leveraging of bacteriocins for use in biocontrol applications. *Psy* B728a, a bean pathogen, produces two toxins that are active against *Psa* BV3 under varying conditions. These include an R-type bacteriocin active against the wild type *Psa* BV3 and an S-type bacteriocin that is only active against strains of *Psa* that have mutations in genes involved in the LPS biosynthesis conferring full resistance to the R-type bacteriocin. To progress the knowledge of this system and evaluate the feasibility of this system for biocontrol applications, two questions were investigated: What receptor or structure does the S-type bacteriocin recognize and is there a fitness cost associated with development of mutation to these toxins.

Twelve mutants resistant to both the R-type and S-type bacteriocins were generated to isolate the mutations causing resistance to the S-type bacteriocin. These mutants were sequenced and aligned with the genome of the parent strain. This revealed a section of about 70 genes that were deleted in nine of the twelve mutants while a tenth mutant had a frameshift mutation in a gene in this area. The remaining two mutants without the region of deleted genes had mutations elsewhere in the genome. From this analysis, the hemolysin secretion/activation, TonB dependent siderophore receptor, *hflB*, and PQQ-dependent catabolism-associated beta propeller genes were identified as candidates for controlling development of resistance to the S-type bacteriocin. Cloning of mutated genes into the wild type background or gene inactivation is necessary to confirm causation of S-type resistance from these genes.

Anecdotal observation and conventional understanding of bacteriocin-related competition networks suggested that some mutant strains should display reduced fitness as a result of the development of their resistance. A growth assay in liquid culture determined that the doubling time for the P1B1 (4.75 hours) and P4G2 (3.44 hours) R- and S-type resistant mutant strains were significantly longer than those of wild type *Psa* BV3, A2 (LPS mutant parent strain), and two other R- and S-type resistant mutants which had doubling times of around 2 hours that were not statistically different from one another. The doubling time of the mutant strains does not appear to be linked directly to whether the strain underwent the deletion of the gene region. While fitness costs in two of the mutant strains were not observed in this experiment, other costs unrelated to growth rate, like increased vulnerability to environmental changes could still have occurred.

All mutants appeared at the same time during generation, while slower growth rates were observed after transfer to fresh KB medium. This suggested that metabolites present from either the lysed *Psa* BV3 cells or the *Psy* B728a supernatant used for mutant selection caused growth rate recovery. In a growth rate assay in liquid culture using supernatant from *Psa* BV3 as well as *Psy* B728a with the R- and S-type bacteriocin deactivated, no growth rate recovery was observed. The mutant strain used in this assay also performed worse in the *Psy* B728a bacteriocin deactivated supernatant compared to its growth in KB relative to its A2 (LPS mutant) parent strain, suggesting decreased ability to grow in nutrient-poor environments.

Because cloning of the genes between the parent and mutant backgrounds has not been completed, finishing this process is one of the first steps that should be taken during future work in determining the genes related to S-type bacteriocin resistance. Increasing the concentration of the antibiotics used in the selection plates as well as ensuring these plates are used immediately after they are made would help to ensure that full screening of *E. coli* takes place, eliminating this

as a possibility for the unexpected recombinant morphology. Should unexpected phenotypes continue to arise in the recombinant strains, this could indicate an interesting response to stress and multiple copies of genes by the strains in use, which could warrant further investigation.

Future work on the causes of the slower growth rate in some mutants could focus on the possible effect that the lysing of cells, whether they are the bacteriocin producing strain or the wild type strain, could have on growth rate recovery. When generating supernatant for resistance testing, mitomycin C was added to stress the producing strain, which encourages bacteriocin production. These cells must lyse to release the bacteriocin, which also releases their cellular contents. The non-resistant cells that die during production of resistant mutants would also release their cellular contents into the culture as they die. Completing a growth assay with induced or lysed cultures could help to narrow down the source of the metabolite responsible for growth rate recovery.

Additionally, future growth assays could be completed at a longer time scale. While the growth rates of most mutants and the wild type strains are statistically the same over the 5-hour timescale in this experiment, growth over a longer time period could show differences in growth rate between the wild type, parent strain, and different genotypes of mutants.

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ACADEMIC VITA

Andrew May

EDUCATION

Penn State University**,** University Park, PA — Graduation: May 2019

Schreyer Honors College

Bachelor of Science – Biological Engineering
(Food and Biological Process Engineering Option)

Minors- Plant Pathology, Environmental Engineering

Study Abroad- Monash University, Melbourne, Australia — *Spring 2017*

HONORS AND AWARDS

Dean's List in all Semesters

Piekert Scholarship in Agricultural and Biological Engineering — *2016-17, 2018-19*
For academic excellence in Biological Engineering.

Rumbaugh Agricultural Leadership Award — *2018-19*
For academic excellence within the College of Agricultural Sciences

Alpha Epsilon Honors Society — *Fall 2017 to Present*
Honors society for Agricultural, Food, and Biological Engineers

Kjelgaard Scholarship for Agricultural and Biological Engineering — *2016-17*
For academic excellence in Biological Engineering

Langdon Learning Endowment — *2016-17*
For academic excellence in Biological Engineering

PRESENTATIONS

Confirmation of Genetic Mutations as the Cause of — *June 2018*
Bacteriocin Resistance in *Pseudomonas syringae* (Poster)
Drew May, Stacey Scott, Kevin Hockett
PSU Plant Biology Symposium: Wild and Tamed Phytobiomes
University Park, PA

Confirmation of Genetic Mutations as the Cause of — *April 2018*
Bacteriocin Resistance in *Pseudomonas syringae* (Poster)
Drew May, Stacey Scott, Kevin Hockett
Gamma Sigma Delta Research Symposium, University Park, PA

Conditionally Redundant Bacteriocins Produced *April 2018*
by *Pseudomonas syringae* (Talk)
Drew May, Stacey Scott, Kevin Hockett
Phage Hunters Research Symposium, Huntingdon, PA
Awarded Best Overall Talk and Poster

Confirmation of Genetic Mutations as the Cause of *April 2018*
Bacteriocin Resistance in *Pseudomonas syringae* (Poster)
Drew May, Stacey Scott, Kevin Hockett
Phage Hunters Research Symposium, Huntingdon, PA
Awarded Best Overall Talk and Poster

Fish Gills: A New Approach to Heat Exchangers (Poster) *April 2016*
Drew May, Kyle McIlroy, Hanna Feldstein
PSU Undergraduate Engineering Expo, University Park, PA

RESEARCH & DESIGN EXPERIENCE

Research Assistant - Biglerville, PA *Summer 2015 & 2016*
Penn State Fruit Research and Extension Center *Winter 2016-17*

- Collected data on tree fruit disease frequency and severity for pesticide efficacy trials and use by local fruit growers.
- Managed and monitored disease in 10 research orchard plots containing 5 different species (apple, peach, pear, cherry, and apricot).
- Isolated, purified, and organized 50 fungal samples in apple storage trials.
- Assisted in training new employees on upkeep and data collection procedures.
- Suggested changes to streamline experimental procedures and minimize waste.

Undergraduate Researcher - University Park, PA *Spring and Fall 2016*
Rosa Lab, Penn State Department of Plant Pathology

- Conducted independent research on the molecular characterization of an economically
- important viral plant disease showing differential symptoms on host plants.
- Documented symptoms of infected plants for disease severity evaluations
- Exercised phytosanitation procedures to prevent viral contamination of samples
- Awarded College of Ag Undergraduate Research Grant ($2000) in Fall 2016

Undergraduate Researcher - University Park, PA *Fall 2017 to 2018*
Hockett Lab, Penn State Department of Plant Pathology

- Conducted independent research on antimicrobial compounds produced by plant pathogenic bacteria for future publication and possible use as a biocontrol agent.
- Utilized molecular techniques to complete a gene transfer between bacterial strains
- Maintained stocks and samples from 7 strains of bacteria
- College of Ag Undergraduate Research Grant ($2000), 3-time awardee

Capstone Design Project - University Park, PA *Fall 2018 to Spring 2019*
Richard Lab, Penn State Department of Agricultural and Biological Engineering

- Designing fermentation system supporting intermittent mechanical milling of high-solids content biomass slurries.
- Gaining experience in system fabrication and optimization.

- Served as communicator and coordinator with sponsor and other groups by sending weekly update emails and organizing meeting times and topics.

LEADERSHIP EXPERIENCE

President – Alpha Epsilon Biological Engineering Honors Society *Fall 2018-2019*
- Selected by faculty and led initiation of new members.

Undergrad Member – University Sustainability Steering Committee *Fall 2018-2019*
- Evaluated proposals for university wide sustainability initiatives at Penn State

President –Undergraduate Plant Pathology Club *Spring 2018-2019*
- Founding Member in Spring 2016
- Web Coordinator in 2016, Secretary in Fall 2017
- Facilitated 100% placement of members with faculty to complete research

Member – American Pytopathological Society Task Force *Spring 2018-2019*
- Designed undergraduate section of organization website
- Helped design undergraduate programming for upcoming meetings

Member – PSU PPEM Departmental Outreach Committee *Spring 2018*
- Coordinated undergraduate, graduate and faculty outreach efforts

Representative – Penn State Ag Student Council *Fall 2017-2018*
- Coordinated undergraduate, graduate and faculty outreach efforts