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TRACKING THE MOVEMENT OF PSEUDOMONAS SYRINGAE STRAINS- ON THE ADAXIAL LEAF SURFACE OF ARABIDOPSIS THALIANA

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TRACKING THE MOVEMENT OF *PSEUDOMONAS SYRINGAE* STRAINS^{-} ON THE ADAXIAL LEAF SURFACE OF *ARABIDOPSIS THALIANA*

by

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ABSTRACT

TRACKING THE MOVEMENT OF *PSEUDOMONAS SYRINGAE*

STRAINS^{-} ON THE ADAXIAL LEAF SURFACE

OF *ARABIDOPSIS THALIANA*

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The University of Texas at Arlington, 2014

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Detection of population density is a form of communication for certain phytobacteria that usually involves the association of small diffusible chemical signals, particularly N-acyl homoserine lactones. It is pondered whether or not this molecule and the phytotoxin, coronatine, play a significant role in the directional motility of the wild type (*Pst* DC3000) and mutant strains (*Pst* DC3118, *Pst* DB29, and *Pst psy/IR*$^{-}$) of *Pseudomonas syringae* pv. *tomato* on the adaxial surface of *Arabidopsis thaliana* leaves. A leaf assay and *in vivo* experimentation by spotting a set of leaves for each bacteria and making an imprint on an agar plate following a designated time-point was conducted to observe for existence of such phenomenon. Patterns of colony formation were noted and distance from the spotting point to the farthest point traveled was measured in millimeters to make a comparison between the mutant strains to the wild type bacterium. Based on

the average distance calculated per time point and for all time points, data suggest that there is not a statistical significance in directional motility between the wild type strain and the mutant strains. Therefore, AHL and coronatine does not seem to contribute to directional motility aside from their standard roles. This determination is not conclusive, however, due to few trials and sample size. Since the objective was also to develop a new protocol, further investigations and studies must be replicated to engender conclusive results and suggestive implications.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Behind the Art of this Experimentation

Many ongoing scientific researches of *Arabidopsis thaliana* have put forth relentless efforts to further investigate about pathogen-plant interactions generally related to hormone signaling pathways, immunity responses, induced stomatal openings, as well as many other biological regulations that take place both within and outside of the plant. However, equal attention should also be directed to understanding more about various factors and mechanisms that affect the way phytopathogens interact with one another to internalize the leaf tissue. Evidence is known about what chemical signals play a role in bacterial communication. However, little is known about how certain patterns of bacterial movement are achieved upon traversing the surface before they enter the interior of the plant and cause an infection. Not much has been mentioned about directionality of bacterial movements themselves *in vivo*.

This research is mainly focused on tracking the movement of four specific bacterial strains of *Pseudomonas syringae* pv. *tomato* (Pst) on the upper leaf surface of *A. thaliana* leaves. Because this particular study has not been conducted before, another objective prior to this experiment is to develop and modify a protocol for future reference. Further progress in this research may help to give a better insight about what other variables play a role in bacterial group responses and how that is required for the bacterium to start its virulent phase as endophytes. Disruption of all bacterium behavior

on the leaf may provide opportunities to control or alleviate plant infection and consequently diminish the impact of diseases on plant productivity.

1.2 Quorum Sensing

Before any further elaboration can be made about the components of this study, the principle of quorum sensing must first be acknowledged. Quorum sensing is a communication system that is often exhibited within particular species of gram positive or gram negative bacteria (Schaefer et al. 2008). Following the detection of a particular threshold in population, quorum sensing exclusively involves a stimulus-response interaction between membrane receptors and various classes of chemical signaling molecules, and this may occur within a species of bacteria or between species of bacteria (Strauss 1999). Such phenomenon allows for a multitude of biological processes and has been thoroughly researched by many scientists. Some activities resulting from quorum sensing include virulence, biofilm formation, antibiotic resistance, and bioluminescence, which was first described in the marine bacterium *Vibrio fischeri* (Cha et al. 1998).

Quorum sensing itself is an important reference behind the action taking place by the model organism of interest: *P. syringae.* Although this study does not require an intricate analysis of all chemical signals involved or any mapping of the pathways and mechanisms taken underway, attaining some basic knowledge of distinct components will be sufficient enough to establish a foundation about quorum sensing.

1.2.1. N-acyl Homoserine Lactones

Studies have shown that plant-pathogenic and plant-associated bacteria are capable of sensing population density through detection of small diffusible molecules known as N-acyl homoserine lactones (AHL). This is an important chemical signal for

bacterial communication in *P. syringae*. When population size reaches a particular threshold, gene expression for production of these chemical signals are positively regulated in response to the accumulation of extracellular AHL from each individual bacterium (Quinones et al. 2003). *Pseudomonas syringae pv. syringae* itself requires AhIR as the regulator and AHL synthase AhII for mediation of the quorum-sensing system (Quinones et al. 2005).

1.2.2. Coronatine

Coronatine (COR), a phytotoxic molecule, is produced by several strains of *P. syringae* (Block et al. 2005). The production and transmission of COR is responsible for the suppression of plant immune and defense systems. In cases of plant contact with non-host bacterial pathogens, a certain bacterial protein is recognized by PAMPS (pathogen-associated molecular patterns) to initiate several mechanisms of defense that include, but are not limited to, stomatal closure and hypersensitive reactions that induce cell death (Lee et al. 2013). Coronatine from *P. syringae* can override this defense barrier and cause devastating outcomes to plant health and longevity in *A. thaliana*. It is unclear whether both AHL and COR play a role in directional motility on the leaf surface. If distinct movement patterns are observed and statistical calculations show a significant difference in terms of average distance traveled between the mutant and wild type strains, future researches can direct new molecular genomic studies that relate to AHL and COR impact on directional motility.

1.3 *Arabidopsis thaliana*

Because of its relatively quick life cycle and ease of maintenance in the laboratory setting, *Arabidopsis thaliana* is usually the chosen plant model for studying genotypic as

well as phenotypic features of plant-pathogen interactions after infection of a phytopathogen has taken place. There are various mutants of *A. thaliana* related to plant defenses, but since this experimentation focuses exclusively on external observations, only the wild type Arabidopsis (Col-0) should be considered for use. The fact that Arabidopsis requires approximately 6 to 8 to complete the entire life cycle allows for a reasonable number of experimental replications in a short time frame.

1.4 Strains of *Pseudomonas syringae*

Four bacterial strains of *P. syringae* was assessed which include *Pst* DC3000, *Pst* DC3118, *Pst* DB29, and *Pst psyI/R-*. *Pst* DC3000 is the wild type strain that attains the ability to reopen stomata upon closure when the plant first recognizes the pathogen (Melotto et al. 2006, Elizabeth and Bender 2007). It is often the choice for studying localized and systemic infections at the genomic level as well as for observing physiological damage that result from virulence at the phenotypic level. *Pst* DC3118 and *Pst* DB29 are mutants of *Pst* DC3000 that do not produce coronatine, and therefore are not capable of reopening the stomata (Melotto et al. 2006, Zeng and He 2007). However, their potential for virulence is no different from *Pst* DC3000 once they are able to penetrate the interior of the host plant. *Pst psyI/R-* is a strain developed by Lenore Price from our lab, which is a double mutant for AHL synthase and AHL receptor (Price and Melotto, unpublished). This simply means that among colonies, the bacterium cannot produce AHL nor respond to AHL. Physiological characteristics of these bacteria as described may or may not have the potential to affect directional motility in any possible way.

CHAPTER 2

MATERIALS AND METHODS

2.1 Preparation of Flats

Flats were usually prepared by first filling each pot in the tray with 1:1:1 ratio of potting soil, perlite, and vermiculite. Perlite and vermiculite are necessary components of the mixture because they allow for proper aeration of the soil and prevent drowning in the event of adding mildly large amount water to the tray on a weekly basis. It is also important that moisture is retained at the surface level of the pots if there happens to be an extreme depletion of water in the tray. The appropriate medium for this matter is a handful of slightly damp vermiculite, which is to be plopped onto the surface of each filled pot. Once this step was done, a large square mesh was placed and pressed above the vermiculite mound to ensure that there were even levels of vermiculite, and the edges of the mesh were then bounded to the pot with a rubber band.

Eight pots per tray were prepared to attain sufficient quantity of leaves for study. Col-0 seeds mixed well in agarose gel were then pipetted to the soil mixture in a fashion depicted in Figure 1, with approximately four seeds per section. The tray, approximately 2 ½ inches in height, is then filled ¼ of the way with tap water. Addition of 300 mL Gnatrol is also a necessary component, which acts as both a fungicide and larvicide to prevent unwanted growth of fungus and gnats in the soil. To initiate homogeneous growth, the entire apparatus was placed in a $4^{O}C$ cold room for three days. The flat was

then removed and placed in a 22^{O}C growth chamber with sufficient lighting for Arabidopsis to continue its growth cycle.

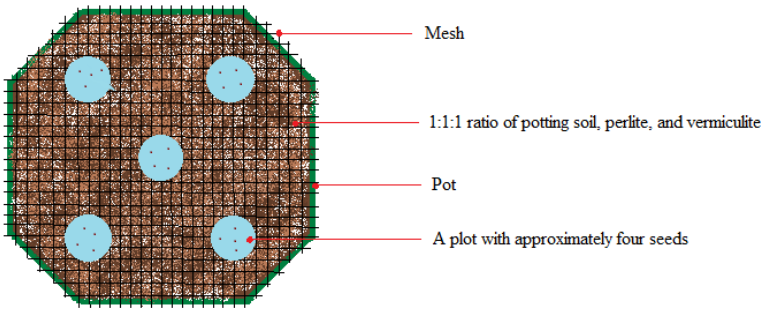

Figure 2.1: Method for sowing seeds (top view). Once Col-0 seeds have germinated within the first or second week, trimming using forceps was necessary to select for the best growing plant for each plot.

2.2 Preparation of Bacterial Inoculum

Separate liquid broth cultures for *Pst* DC3000, *Pst* DC3118, *Pst* DB29, and *Pst psy/IR*$^{-}$ were prepared by adding one isolated colony of each strain to their designated flask, each containing low salt lysogeny broth (LS LB) and f rifampicin (Rif100). The liquid broth culture for *Pst* DC3118 requires an addition of kanamycin (Kan50). Both rifampicin and kanamycin are antibiotics that selectively inhibit growth of bacterial strains other than the specified strains of *P. syringae*. All four flasks were then placed in a 30.0^{O}C incubator with 250 g and left for 15 hours.

With the use of a spectrophotometer set to a wavelength of 600 nm, an optical density check was then performed to ensure that each bacterium had a growth between 0.6 and 1 O.D. If satisfactory OD measurements were obtained, the liquid cultures were then centrifuged for 20 minutes with settings of 3000 g and 20^{O}C. The supernatant from each centrifuged vial was disposed, leaving behind clumped sediments of raw bacterial culture. Depending on the specific O.D. obtained, a specific volume of distilled water must be added to dilute the concentration to a desired level using the equation C_1V_1 =

C_2V_2. The initial aim was to utilize each bacterium at a concentration of 10^8 CFU/mL, which correlates to an O.D. of 0.2. Concentrations were then doubled in the last couple of trials to see if more bacteria stayed on the plate.

2.3 Leaf Assay

In this leaf assay, leaves of *A. thaliana* were detached to perform the experiment. Since leaves depend on water transport from roots to the vascular tissues of leaves, there must be a suitable method to keep the leaves alive for the duration of experimentation. A petri dish with a circular cut-out of an underpad was moistened with sufficient distilled water to create a water reservoir.

Once the plants have matured from 4-5 weeks, they were used for experimentation. This was to ensure that the leaves themselves are not too young or too old. The middle layer leaves of *A. thaliana* were preferable subjects to use since they were neither large nor small in length and width. Using sterilized scissors to minimize any closure or damage to the vascular tissues, the leaves were detached approximately 1½ cm from the base of the leaf (Figure 2.2). Each leaf was then washed in 70% ethanol for 30 seconds and then washed with distilled water for another 30 seconds. Once dried, leaves were carefully adjusted to stand upright so that water will be able to enter the vascular tissue at the base of the leaf petiole. A set of leaves were prepared for each bacterial strain.

Figure 2.2: *Arabidopsis thaliana* leaf detached approximately 1½ cm from the base.

2.4 Spotting and Imprinting

Designated plates with the appropriate amounts of antibiotics specified in section 2.2 were left outside for five days to relieve the media from any condensation. When placed in a cold setting, a layer of moisture can usually be seen on the lid and sometimes on the media itself. Moisture of any sort on the media as well as the leaves is undesired since that will allow bacteria to spread in all directions when the leaves are printed on the agar media. Therefore, it is essential that the media and leaves are dry as possible to refrain from obtaining inconclusive results.

Using a pipet, 1 µL of bacterial solution (Figure 2.3) was extracted and spotted on each side of the midvein near the base of the leaf. The entire apparatus can be seen in Figure 2.4. The entire process was carried out at room temperature under a ventilating hood, and the air blowing from the back wall was utilized to dry the spots at a faster rate (Figure 2.5). Once dry, the leaves were to be evenly pressed on the agar media for 30 seconds and lifted quickly starting from the petiole. Eight time points were taken with 30 minutes being the first and the rest followed by half hour intervals. Figure 2.6 depicts the layout of the experimental design.

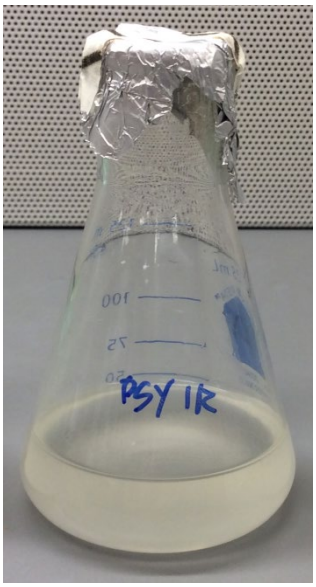

Figure 2.3: Example of a spotting inoculum for psy/IR- which includes bacteria attained from the liquid culture after centrifugation and a specific volume of water.

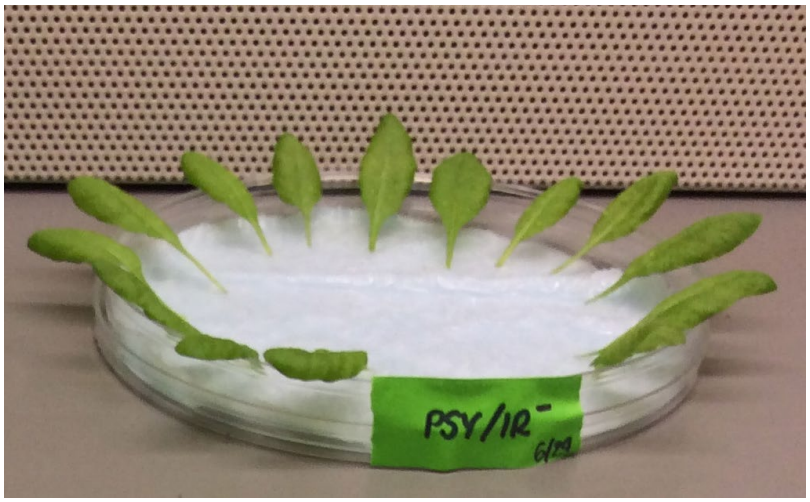

Figure 2.4: Representation of a leaf assay near the ventilating hood with blowing air.

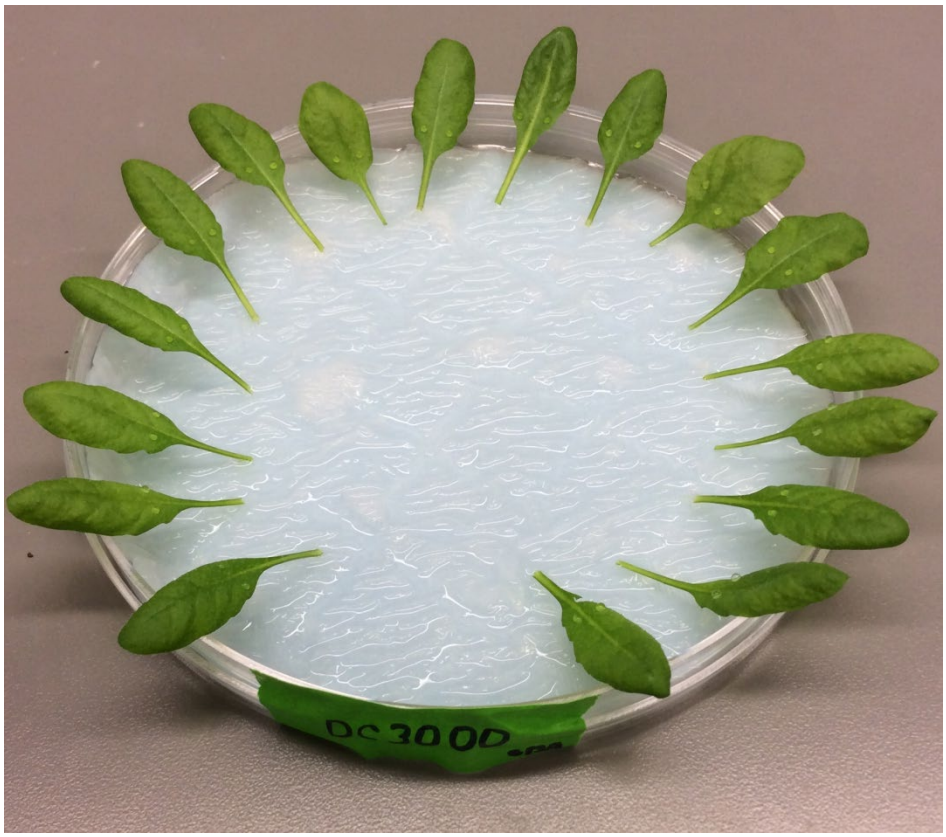

Figure 2.5: A close up view of the leaf assay with spotted leaves near the base of the leaf on each side of the midvein.

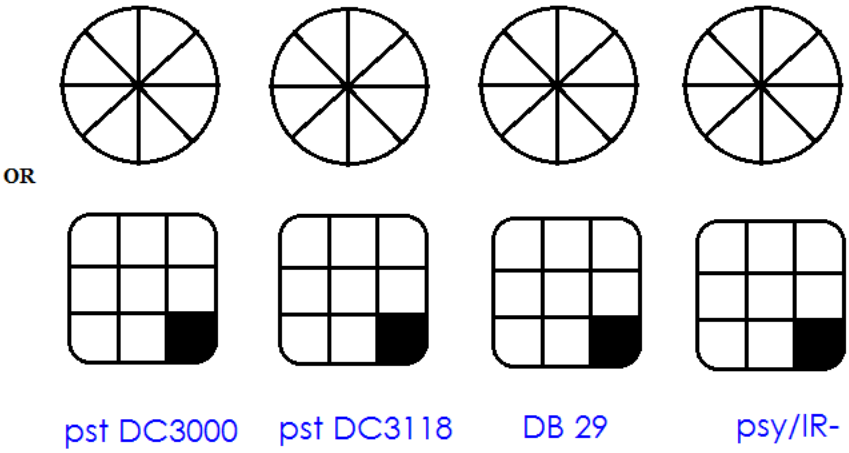

Figure 2.6: A representation of the experimental apparatus where each circle or square is a plate filled with agar media and respective antibiotics. Since eight time points were needed, each plate was evenly divided into eight sections for leaf imprinting.

2.5 Statistical Analysis

Method of recording distance was taken by measuring bacteria colony formation from the point of spotting to the farthest point traveled in a straight vertical direction (both marked in the agar media with a sterilized pipet tip). Data obtained for each individual bacterium per trial was then averaged to obtain a mean value. Using a two-tailed student's t-testing based on total number of reliable samples from each experimental trial, mean values for distance were analyzed for statistical significance where calculated P-values greater than 0.05 shows insignificance and P-values less than 0.05 indicates a statistical significance. All observations for differences in average distance were done in comparison between the mutant strains and *Pst* DC3000.

CHAPTER 3

RESULTS

3.1 Colony Count and Average Distance Traveled

Tables 3.1 and 3.2 summarize the approximate number of bacterial colonies per time point and the average distance traveled per time point, respectively. Though values in Table 13.1 are not involved in any calculations, they do give a relative comparison between the mutant strains and *Pst* DC3000 in terms of which strain seems to grow faster. Measurements recorded in Table 3.2 may possibly have two values followed by a (+) or (-) sign to indicate location of the bacterial colonies relative to the point of spotting. A (+) sign typically means that the bacteria traveled in an upward direction above the point while a (-) sign is an indication that the bacteria traveled below the point towards the base of the leaf. Before calculating average distance per time point and for all time points, both values will be additive to attain a value for the entire distance traveled. Sections of the media with dense mold growth were disregarded since there is no clear indication of bacterial colony formation. Though it was anticipated that there would be 24 samples (8 sections x 3 trials) per bacteria, mold infestation has reduced the number of samples for each strain and varies as well.

Figure 3.1 is a graphical representations of average distance traveled per time point. Error bars attained from standard error values are shown to establish a relationship between mutant strains and *Pst* DC3000 in terms of any significant differences. P-values

were then attained through a two-tailed student's t-test calculation. Each P-value represents a pair-wise comparison between *Pst* DC3000 and each mutant for each time point (Table 3.3). When comparing, any values less than 0.05 indicates that one bacterium has traveled faster than the other. Thus, it is concluded that there is significant difference in distance travelled between the mutants and wild type bacteria.

Table 3.1: Colony count for each strain per trial depending on the concentration utilized.

	Number of Bacterial Colonies Per Time Point							
Trial 1								
Bacterium (*P. syringae* pv. *tomato* strains) 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minutes	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	11	2	0	0	0	0	0	0
DC 3118	3	1	0	0	0	-	-	-
DB 29	3	0	0	0	0	0	0	0
psy/IR$^-$	2	0	0	0	0	0	0	0
Trial 2								
Bacterium (*P. syringae* pv. *tomato* strains) 2 x 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minutes	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	75	16	8	5	2	-	0	-
DC 3118	120	81	55	25	16	-	-	2
DB 29	45	-	6	-	0	0	0	0
Psy/IR$^-$	110	125	24	22	35	-	-	-
Trial 3								
Bacterium (*P. syringae* pv. *Tomato* strains) 2 x 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minutes	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	85	-	-	-	0	0	0	0
DC 3118	51	78	52	-	-	-	-	-
DB 29	20	39	11	-	-	-	-	-
Psy/IR$^-$	140	116	92	-	-	-	-	-

(-) indicates mold infestation to where no colony formation could be counted.

Table 3.2: Record of measurement from the spotting point to farthest distance traveled.

	Average Distance Traveled Per Time Point (in mm)							
Trial 1								
Bacterium (*P. syringae* pv. *tomato* strains) 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minute	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	1.60	1.00	0	0	0	0	0	0
DC 3118	1.00	0.85	0	0	0	-	-	-
DB 29	1.40	0	0	0	0	0	0	0
psy/IR$^-$	1.20	0	0	0	0	0	0	0
Trial 2								
Bacterium (*P. syringae* pv. *tomato* strains) 2 x 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minutes	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	1.90+ 0.50 -	0 + 0.60 -	2.00	0.80	0.40	-	0	-
DC 3118	3.00	2.50+ 1.60-	2.00	1.60	1.50	-	-	0.70
DB 29	2.20+ 0.7 -	-	1.40	-	0	0	0	0
psy/IR$^-$	1.70+ 0.70-	2.40+ 0.5-	2.30+ 0.5-	2.40+ 0.4-	1.80+ 0.6-	-	-	-
Trial 3								
Bacterium (*P. syringae* pv. *tomato* strains) 2 x 10^8 CFU/mL	30 minutes	1 hour	1 hour & 30 minutes	2 hours	2 hours & 30 minutes	3 hours	3 hours & 30 minutes	4 hours
DC 3000	2.40	-	-	-	0	0	0	0
DC 3118	2.5	2.6	2.8	-	-	-	-	-
DB 29	1.70	2.30	0.60	-	-	-	-	-
psy/IR$^-$	2.80 0.7	2.75 0.5	2.00 0.5	-	-	-	-	-

(-) indicates mold infestation to where no colony formation could be counted

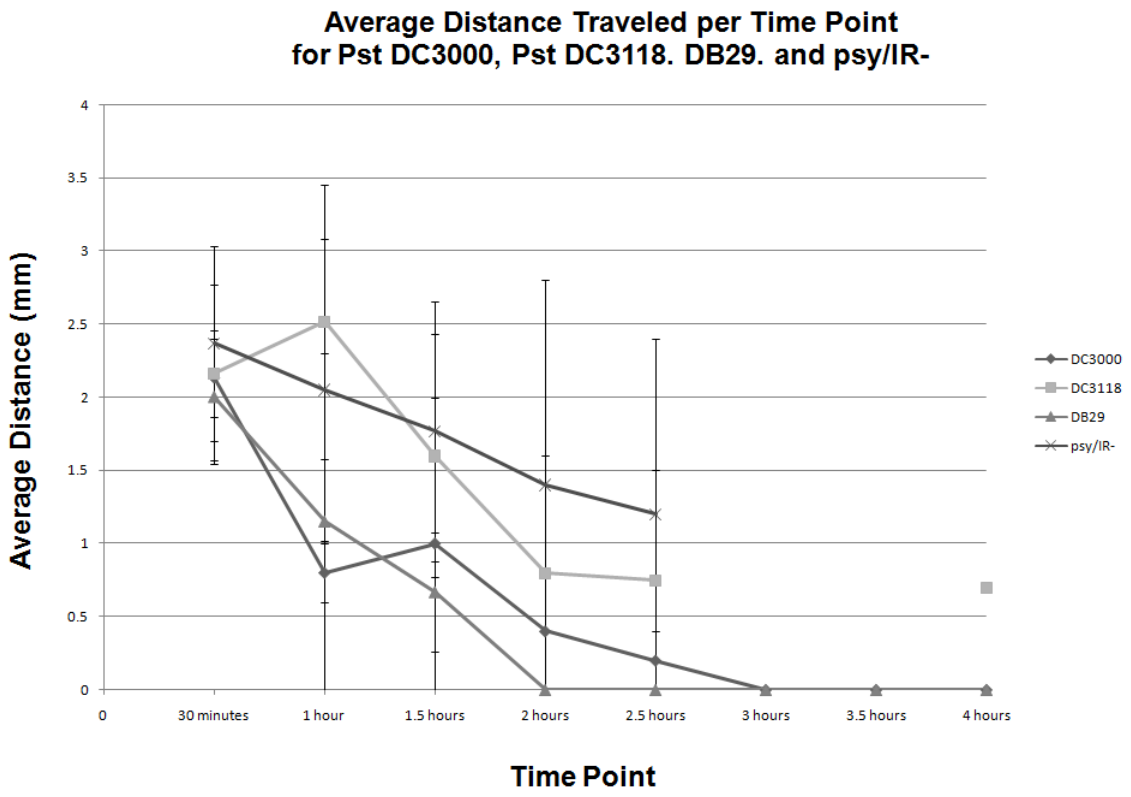

Figure 3.1: Line graph depicting average distance traveled (in mm) by *Pst* DC3000, *Pst* DC3118, *Pst* DB29, *Pst psy/IR*$^{-}$ for all time points with standard error bars to indicate any significant differences between two or more strains. Each time point is represented by half- hour intervals.

Table 3.3: Summary of P-values calculated between *Pst* DC3000 and each individual mutant to assess if average distance traveled between two strains show any statistical significance.

	Pst DC3000 and Pst DC3118	Pst DC3000 and Pst DB29	Pst DC3000 and Pst psy/IR-
30 minutes	0.962	0.814	0.761
1 hour	0.255	0.793	0.419
1.5 hours	0.698	0.739	0.614
2 hours	0.698	0.422	0.563
2.5 hours	0.369	0.369	0.326
3 hours	-	-	-
3.5 hours	-	-	-
4 hours	-	-	-

(-) indicates that a p-value could not be obtained due to insignificant data points where either there were no growth (distance = 0 mm) or that growth could not be discerned due to interfering mold growth.

3.2 Colony Growth of Bacteria from Leaf Imprinting

Following a series of trial and errors, the first protocol was adjusted to using bacterial concentrations of 10^8 CFU/mL for each strain. It is apparent in Figure 3.1 that multiple colonies formed from the *Pst* DC3000 leaf imprint while there were only 3

colonies for *Pst* DC3118, 3 colonies for *Pst* DB29, and 2 colonies for *Pst psy/IR*$^{-}$ within the first 30 minutes of spotting. The second time point (1 hour) shows significant reduction in colony formation where only two was is visible for *Pst* DC3000, 1 for *Pst* DC3118, and 0 for both *Pst* DB29 and *Pst psy/IR*$^{-}$. Any other time points beyond show no presence of growth. Unusual cases of excessive mold formation can be seen in the plate of DC3118 in Figure 3.1. This picture in particular was used because a subsequent pattern could be deduced from the first five time points.

To observe if more bacteria would stay on the leaf within the first 30 minutes, the initial concentration was then doubled to 2.0 x 10^{8} CFU/mL for the remaining trials. A depiction of growth using this bacterial concentration can be seen in Figure 3.2. Generally, more colony formations were present within the first 3-5 time points; and after the fifth time point, there was an absence of colony formation.

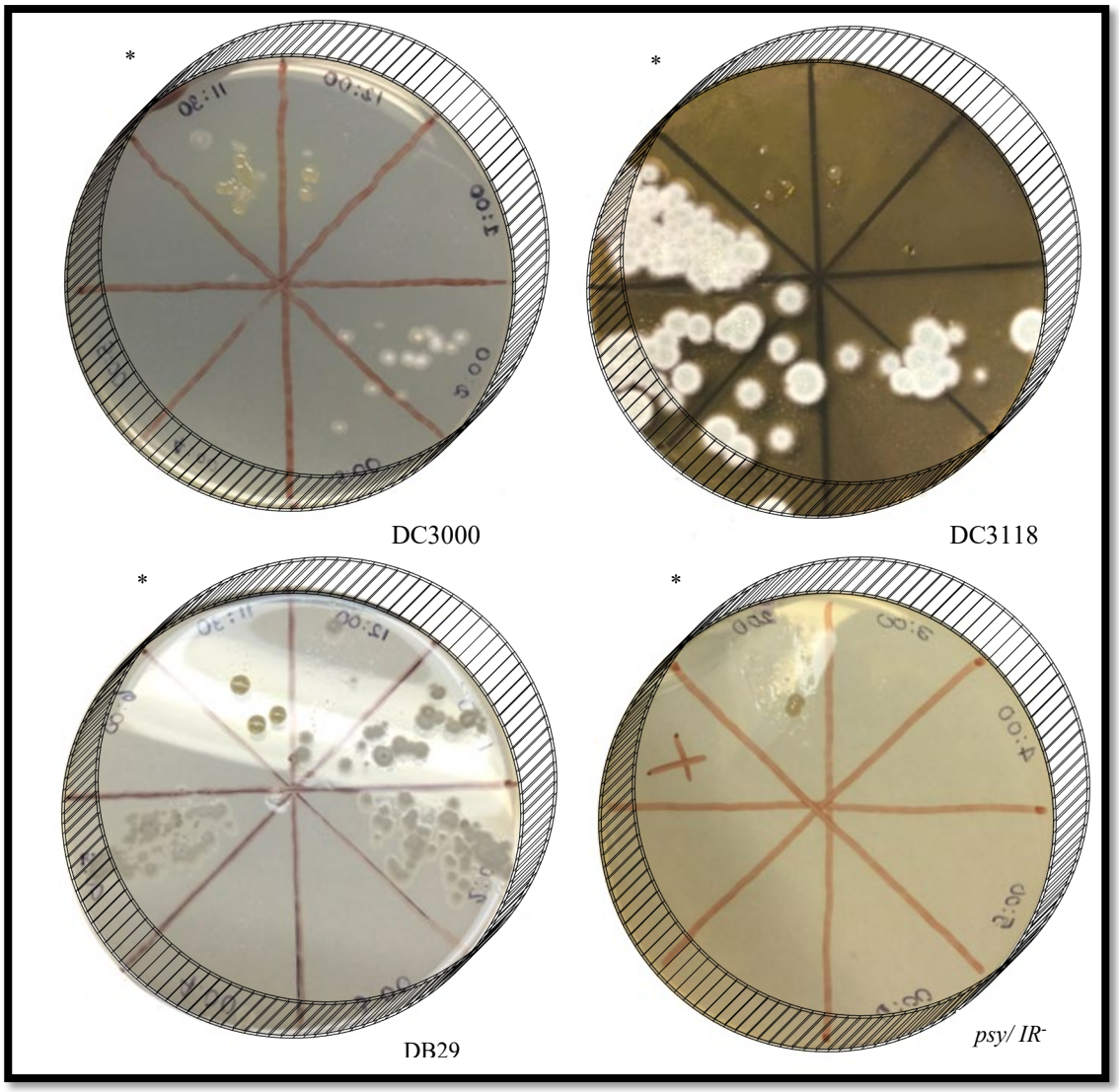

Figure 3.2: *Trial 1* using 10^8 CFU/ mL (O.D. = 0.2) per strain and imprinting for every hour from time point 2 and onward. Yellow forming dots are a sign of bacterial growth while white specks are mold growth and should be disregarded.
* indicates the start of the first time point (30 minutes).

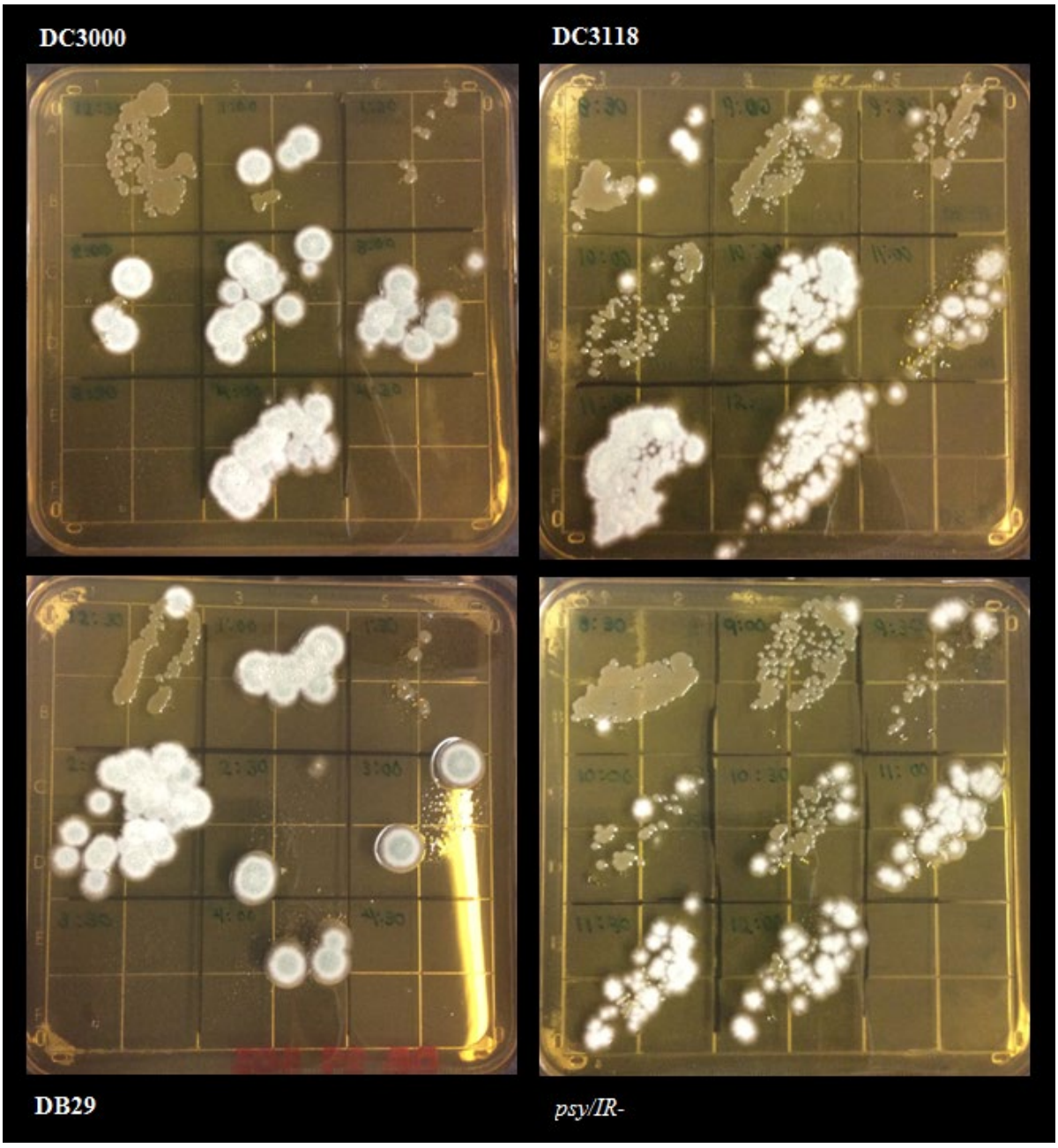

Figure 3.3: Best photos of replicates chosen from Trials 2 and 3 for each strain. Despite instances of mold infestation here and there, certain patterns of bacterial colony formation could be discerned.

CHAPTER 4

DISCUSSION

4.1 Observations in Movement Patterns

4.1.1. Pst DC3000

DC3000 seems to have a very rapid and heterogeneous spread (distinct colonies dispersed) throughout the leaf in the first half hour after the leaf designated for this time period has been spotted. In one to two hours that follow, colonies can be seen to be localized in distinct groupings, sometimes near the base of the leaf or at times near the edge of the leaf. Most important, the number of colonies seem to disappear more than other mutants by the second hour. In trial and error sessions as well as in the official trials, time points near the end of experimentation always seem to show less than 10 colonies. Observations may not be a surprise since DC3000 is indeed one of the most virulent strains of *P. syringae*.

4.1.2. Pst DC3118

DC3318 begins growth with a rapid homogeneous growth (a sheer layer with no individual spaces in between). It was observed that either the left or side of the leaf seems to "lead the movement" around the edge of the leaf. The second time point generally shows movement dispersal with a clearance in the center of the leaf. Progressing into the one and half hours to two and half hours, there is a gradual disappearance of growth in the medial portion of the leaves, and the numbers of colonies around the edges seem to get smaller.

4.1.3 Pst DB29

Interesting about this particular strain is the way that colony formation travels along the edges of the leaf on the first time point and there is a cleared path along the midvein. Colonies can be distinctively seen (not clumped together) and by the third time point, relatively few colonies are seen near and around the edges.

4.1.4 Pst psy/IR$^-$

Patterns of movement for this strain are similar to that of *Pst* DC3000. However, unlike the wild type and other mutant strains, this bacterium can remain far at the top of the leaf starting with the first hour and can be seen by dense conglomeration of colonies. 30 minutes later, it was observed that *Pst psy/IR*$^-$ travel near the bottom half of the leaf. Such observation explains for the generally large average distance over time seen by the steep slope in Figure 3.1. It is unclear at this point whether the movement patterns observed for all strains were a matter of randomness or was associated with a distinct interaction with the chemical or structural properties of the leaf which allowed for a particular path to be favored.

4.2 Interpretation and Analysis of Numerical Data

Overlapping error bars shown in Figures 3.1 implies that there is not a significant difference between *Pst* DC3000 and mutants due to AHL and coronatine's effect on average distance traveled by the strains on the adxial leaf surface of *A. thaliana*. P-values presented in Table 3.3 from the two-tailed student's t-test (all greater than 0.05) clearly show that the results are not statistically significant. It could be further concluded that observed data are rather explained by chance alone. Here, we fail to reject the null, that there is not a relationship between two or more of the experimental strains in terms of

which travels faster than the other(s). Otherwise, it was initially hypothesized that since *Pst* DC3000 was the virulent strain, and they should travel less far in oppose to other mutants that may linger farther and longer on the adaxial surface before possibly making way to stomatal entrances.

The fact that the average distance traveled for *Pst* DC3000 and *Pst* DB29 is nearly the same as represented within the first one and half hours (Figure 3.1) is concerning because *Pst* DC3000 should have the better advantage of entering the stomata earlier since they are capable of producing coronatine. However, such graphical result merely indicates that there is no significance of coronatine on the role of directional motility. But since this experimentation is mainly focused on observational comparison among different strains of *P. syringae*, there were really no expectations as to which strain should have traveled farther or stayed longer on the leaf. Implications could have been more justified if data was available for *Pst* DB29 so that a true comparison could be made with each of the four strains at the second and two-and-half time point.

4.3 Conclusion

Based on the statistical results attained from this study, it can be said that AHL and coronatine may not seem to influence directional motility aside from their usual biological role. Moreover, data implies that movement is more of a random nature than structured. Such conclusion is peculiar since in most living organisms, there is often a sense of directional pattern associated with molecules unique to that organism or group of organisms. In plants, auxin accumulation in cells of the stem away from light sources is responsible for the directional phenomenon of phototropism. This is essential for the plant to maximize its potential for photosynthesis and access to nutrient availability.

For an example in animals, ants communicate through chemical secretions known as pheromones by stroking each others' antennae. This method not only allows the ants to "recognize" each other within the same colony, but also to lead each other to the source of food and back home. The same principle may be related to bacterial movement, in this case, *P. syringae*. It is well understood that AHL and coronatine are directly related to signaling recognition; however, more investigations are needed to be carried out to understand how AHL and coronatine facilitate the directional patterns observed in this experiment. Results are not fully-justified and conclusive since few trials were done and errors occurred during the course of experimentation.

Errors in the development of the protocol are highly responsible for the inconclusive results obtained throughout the course of experimentation. Future improvisations are needed to achieve reliable data. One major error was leaving the plates with rifampicin out to dry for five days. It was acknowledged that half a day should have been the maximum limit else the antibiotic loses its potency. This could have been the major cause of mold infestation or the presence of other epiphytic bacterial species growing on the plate. Growth of mold and other bacteria may also be due in part to the manner in which leaf sterilization was handled.

Cleaning leaves is a difficult step as this experimentation involves leaf surface imprinting. In nature, epitphytes often depend on nutrients that originate below the cuticular membrane and up to the surface (Lindow et al. 17). If the leaves are kept too long in the cleaning solvents prior to *P. syringae* spotting on the surface, many natural substances of the leaves may be washed away and possibly affect movement and quorum sensing among the bacteria. However, if the leaves are washed too quickly, growth of

other bacterial strains may be imprinted on the agar and affect results if the antibiotic happens to lose potency. A sufficient amount of time designated for ethanol washing and autoclaved distilled water washing must be considered to keep the leaves sterilized while at the same time retain the integrity of their natural properties.

Damage to the leaf both physically (such as when using forceps) and chemically will likely affect interaction of the bacteria on the leaf, as it is the structural pilli of *P. syringae* that allows for rapid adhesion to the surface within the first hour of contact (Romantschuk et al., 1993; Suoniemi et al., 1995). That is why the protocol includes leaves and media to be as dry as possible because water influences flagellar motility and enhance survival on leaves (Haefele and Lindow, 1987). But the fact that early disappearance of bacteria noticed in some replicates raises a concern of what is actually occurring during the course of experimentation. One thought is that with the concentration of bacteria used, they did not survive long enough over time. One study of *P. syringae* on external bean leaves shows that survival and epiphytic fitness in 40% of 82 studied strains were reduced their ability to tolerate stresses related to dry bean surfaces such as high light or UV conditions (Beattie and Lindow 1994). The same idea may apply for *P. syringae* on Arabidopsis leaves. This experimentation was carried out under room temperature with fair amount of light. Other conditions could be tested to see if that will affect directional motility and longevity in any particular manner.

Another explanation for the disappearance of bacteria lies in the fact that the strains could have entered the stomata either by making their way beneath the leaf surface or traveling into damaged leaf surfaces such as broken trichomes (leaf hairs). The best way to check this is to utilize the plating technique. Traditional inoculations to infect *A.*

thaliana often use the plating technique to determine bacterial population size within leaves. If this step was to be further taken, leaves of the assay should have been drilled, serially diluted and plated on a separate plate for each strain to confirm this thought. However, time constraint did not permit the plating technique to be used.

Corrections to any issues of the protocol mentioned above would greatly improve the outcome in determining patterns of directional motility in not just for *Pst* DC3000, *Pst* DC3118, *Pst* DB29, and *Pst* psy/IR- but for many other various strains of *P. syringae* as well. Future directions, if this experiment was to be further elaborated, one may look towards directional motility in response to humidity levels, presence or absence to light, and many other environmental stresses as well. Again, the major goal that this experiment strives to achieve is to assess the route method of *P. syringae* and hope that in future experiments, more can be said about the complete mechanism that tie signaling molecules like AHL and coronatine to directional movement towards sites for penetration on the leaf surface such as stomatal pores. Infection is not caused by just one bacterium alone, but by a conglomeration of single-celled individuals that work through a communication system to bring down a plant's defense system and result in virulence. If there was a proper method to control or disrupt bacterial communication before any arrival can be made on plant entry surfaces, this will greatly improve plant productivity and reduce plant infection and diseases to a minimum.

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BIOGRAPHICAL INFORMATION

Vincent Nguyen was born and raised in Arlington, Texas where much of his childhood was spent watching science shows for young viewers on television. *The Magic School Bus*, *Bill Nye the Science Guy*, and *Zoboomafoo* are some examples of inspiring series that sparked Vincent's sense of interest in the life sciences at an early age. Currently, he is attending the University of Texas Arlington to pursue a degree in Biology, as Bachelor of Science, and minoring in Psychology as well. Vincent had the opportunity to be a student of Dr. Maeli Melotto for a Cell Molecular Biology course back in 2011 and later on became an official member of her plant pathology research lab in 2013. His current undergraduate research project is mainly centered on tracking the movement of *P. syringae* on *A. thaliana* leaves with the influence of Dr. Melotto herself and graduate student, Debanjana Roy. This research is an invaluable experience to Vincent, because not only does it allow him to put knowledge into application, it is a chance for him to express creativity through development of a unique protocol that has never been followed by previous undergraduate members. Through this involvement, Vincent realizes that scientific research is not just about analyzing quantitative measures to reach a conclusion, but is also a work of art. Vincent looks forward to present his subject as part of the Honors Thesis at the FALL 2014 HRS. Upon graduation, Vincent wishes to continue his education in medical school and hopes to become a primary care physician in the near future.