Letters in
General Microbiology

The Effect of Bioaugmented Soil on the Pathogenicity of *Pseudomonas syringae*

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Abstract

It has become of recent interest to study the plant-pathogen interaction as a way to prevent devastating agricultural epidemics. One model system for this study is the interaction of *Arabidopsis thaliana* and *Pseudomonas syringae.* The interaction has been extensively studied; however, practical methods to increase plant resistance to pathogens have not been widely studied. This study looks to investigate this by comparing the effects of pathogenicity in plants sown in bioaugmented soil. Studies have shown that bioaugmentation has beneficial effects regarding growing stronger and healthier plants. Here, Three different inoculation techniques were used to determine if a correlation between soil type and the resistance to infection. Based on the results, it is difficult to say whether or not there is in fact a relationship due to a low replicate number as well as other errors.

Introduction

The study of plant-pathogen interactions has recently become a topic of interest as a way for controlling or avoiding agricultural epidemics (Morris, 2008). A model pathosystems that is routinely studied is the *A. thaliana* - *P. syringae* relationship (Ishiga, 2011). *A. thaliana* has played an important role in investigating plant defense mechanisms (Hagemeier, 2000). One gram negative bacterium that infects *A. thaliana* is *P. syringae. P syringae* enters *A. thaliana* through natural openings, such as stomata or wounds, where it then multiplies in the apoplast to cause disease (Ishriga, 2011). Plant death and growing decay are signs that *P. syringae* has successfully infected (Morris 2008). *A. thaliana* responds by closing the stomata in order to limit entry (Baltrus, 2011).

Although the response of *A. thaliana* to *P. syringae* has been studied extensively, ways to enhance the defense mechanisms have not been fully developed. Recent studies have focused on methods that can be used commercially as a way to increase the health of the plants. One such method is bioaugmentation, which is the artificial addition of microbes to soil (Unno 2005). It is known that high densities of microbes are located in the rhizosphere, the region of soil that surrounds the plant's roots (Unno 2005). One microbe that has been studied is *Bacillus amyloliquefaciens.* This microbe uses organic substances that emanate from the roots, such as sugars and polysaccharides, and transform them to an organic carbon source that is readily available to be used by the plants (Unno 2005). *B. amyloliquefaciens* also increases the phosphotase activity within the rhizosphere, which is an important enzyme to maintain plant nutrition (Unno, 2005).

Bioaugmentation of soil has already proven to be beneficial in growing healthier and more rigorous plants. However, the effects of bioaugmentation in regards to pathogenicity remain unknown (Unno 2005). This study investigates this subject to determine if bioaugmentation provides other benefits. We hypothesize that bioaugmentation of soil with *B. amyloliquefaciens* will increase the efficacy of the plant's defense mechanisms by comparing the *A. thaliana – P. syringae* model interacton in both commercial soil (CS) and bioaugmented soil (BS).

Materials & Methods

Plant Material, Bacterial Strain and Growth Conditions

A. thaliana was planted in CS or BS growing trays within a greenhouse under the following conditions: 12-h photoperiod at 25°C. The concentration of *B. amyloliquefaciens* in BS was 10^6 CFU/mL (as per Dr. Simeon Kotchini, Rutgers the State University of New Jersey, Camden). Twenty-four days after sowing, individual plants from both CS and BS were transferred to plastic pots (1 plant/pot; 3x3 inches). Contamination of water sources was prevented using barriers in the plant basin.

P. syringae strain was originally cultured in liquid Kings B medium at 27°C on an oscillatory shaker set to

200 rpm (Zhang 2009). It was then plated on solid Kings B medium agar plates and stored in an incubator at 30°C.

Plant Inoculations

Colonies on the agar plates were brought into suspension using water. Based on previous studies, the optimal OD600 value of 0.2, or 2.0 x 10^{8} $^{cells}/_{mL}$, was determined using a spectrophotometer (Ishiga 2005). The dilution was then brought to 250 mL to use for inoculation.

Four plants from CS and BS were dip-inoculated 7 days after transplanting. Each plant was submerged for ten seconds in the suspension and quickly placed back into the corresponding pot. The soil was covered to prevent contamination of *P. syringae* into the soil (Ishiga 2005). Four more plants from CS and BS were dip-inoculated with deionized water as a control

Three more plants from CS and BS were inoculated using a pipette 21 days after transplanting. The 30 µL solution of 1:10 Tween 80 in the bacterial suspension was directly placed on individual leaves (Cintas 2002). Deionized water was inoculated into three other plants from CS and BS as a control.

A final method of inoculation was used to test the viability of *P. syringae*. A 0.2 cm diameter syringe filled with 0.05 mL of the bacterial suspension was used to directly inject individual leaves from plants in CS and BS 21 days after transplanting (Ishiga 2011). The leaves that were inoculated were distinctly marked and labeled. For the control, deionized water was injected into the leaves that were not infected.

Detection of Bacterial Infection on Plant

Plants were analyzed every 4 days post inoculation (pi). The extent of symptoms was determined by the presence of leaf deterioration, in regards to structure, texture, and color, as well as the formation of necrotic lesions (Hagemeier, 2000). As the plants began to flower, the length of the stem for each plant was compared.

Results

To test the hypothesis that *A. thaliana* planted in BS would provide better resistance to infection of *P. syringae*, we examined the plants qualitatively and quantitatively. First, the physical characteristics of the plants were observed in all three inoculations (seen in Supplement A). Figure 1 shows one BS and one CS plant 4 days and 19 days after dip inoculation. The plant in the CS showed slight yellowing of the leaves. It is clear to see that the selected plant in BS was successfully infected, as there is yellowing of the leaves with leaf deterioration

Next, the stem lengths of the plants for both the dip and pipette inoculations were measured. The stem lengths for each plant 4 days and 19 days pi are in Supplement B. This data was then compared to each other, as seen in Figure 2. A 2-tailed T-Test was performed on the data and each situation yielded a p value much greater than 0.05. This indicates that there is no correlation between bioaugmentation of soil and whether or not a plant is more susceptible to infection.

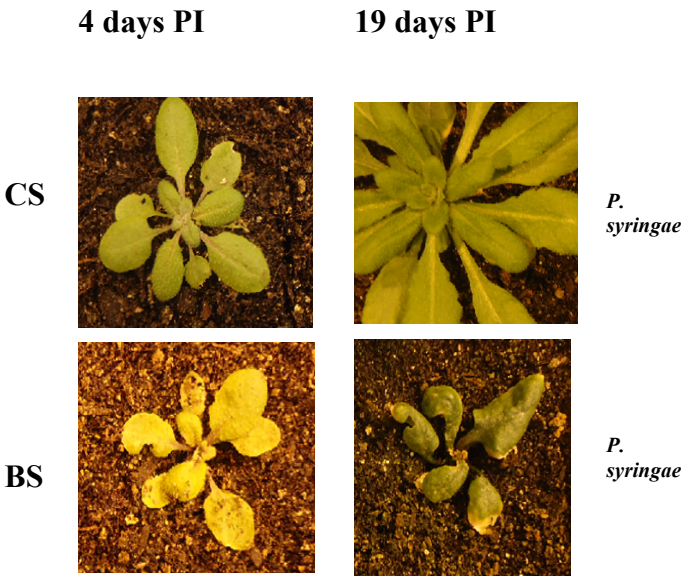

Figure 1 shows the phenotypes for each condition when inoculated via dipping; BS or CS and deionized water or *P. syringae.*

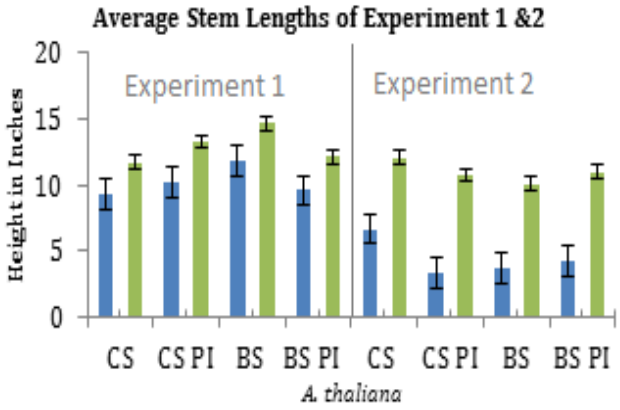

Figure 2 Averages of stem lengths per plant and soil type with y-error bars. Blue and green bars represent measurements taken at days 4 and 19, respectively. Experiment 1 refers to the dip inoculation method while Experiment 2 refers to the pipette inoculation.

Discussion

Based on the phenotypic data and the value of the 2-tailed T-Test, there is no correlation between plants sown in BS compared to those in CS in regards to infection. A conclusion should not be drawn based on this data alone.

The data is provisional as there were few subjects in each condition with only single rounds for each inoculation technique. Sufficient time for testing replicates and secondary inoculations of *P. syringae* should be performed before any conclusion is drawn.

There are additional variables that can account for the observed results. Even though all of the plants were sown at the same time, each inoculation technique was performed at different stages of the plants life. The stage of life the plant is in may have different effects on the pathogenicity of *P. syringae.* It is possible that the plants were not fully inoculated since only one inoculation was done per plant. Based on the results, the syringe inoculation proved to be the most effective method, although not perfect. Infection occurred quickly in the inoculated leaves; however, new leaves replaced and overran the ones that died off. In future experiments, inoculation via syringe or vacuum could be more advantageous and lead to a higher rate of infection. Also, numerous inoculations could yield better results.

Finally, measuring stem length as a determinant for infection proved to be faulty. The leaves, not the stems, of the plants were inoculated so there was no visible sign of infection on the stems. For future research, the part of the plant that is inoculated should be quantitatively analyzed. If the stem is directly inoculated, the stem length would be an appropriate was to measure pathogenicity and vice versa with the leaves.

Acknowledgements:

The study was performed as part of the course requirement for General Microbiology Laboratory at Rutgers University – Camden. We would like to thank Dr. Kwangwon Lee for providing the *P. syringae* strain and Dr. Simeon Kotchoni for providing the *A. thaliana* and *B. amyloliquefaciens.*

References:

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**Supplemental Data

Supplement A: *A. thaliana* pi for all three inoculation techniques.

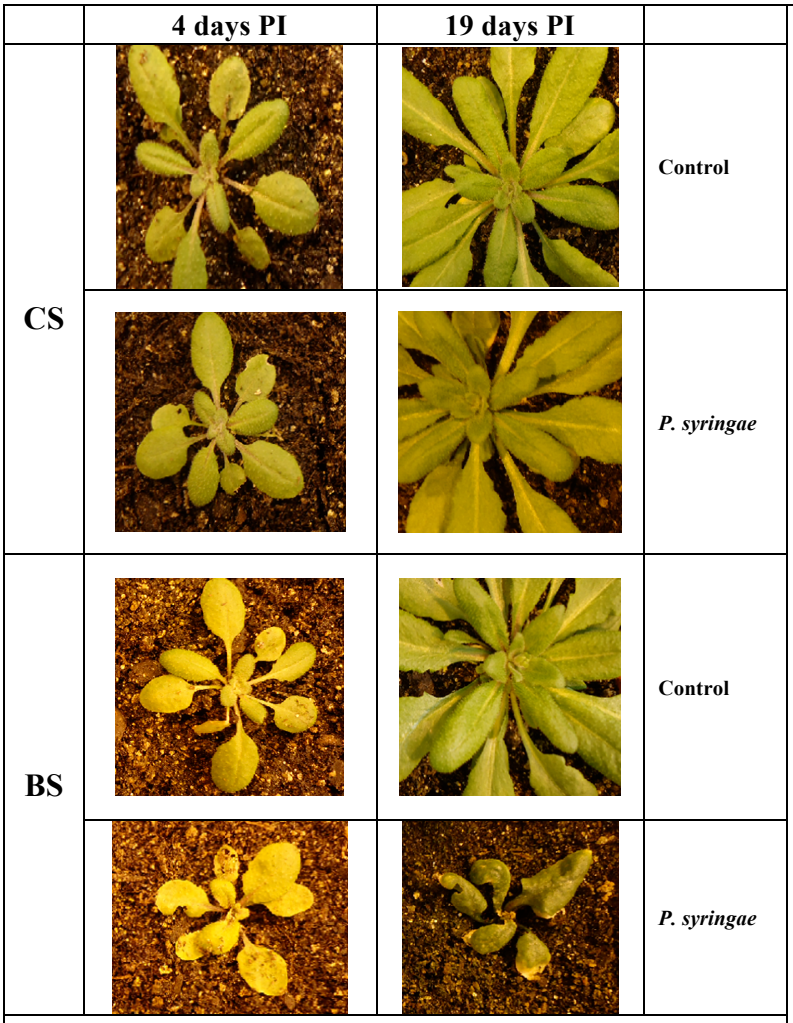

Figure A1: A. thaliana 4 days and 19 days after dipping inoculation with either deionized water or *P. syringae.* There are no significant differences between soils in regards to pathogenicity.

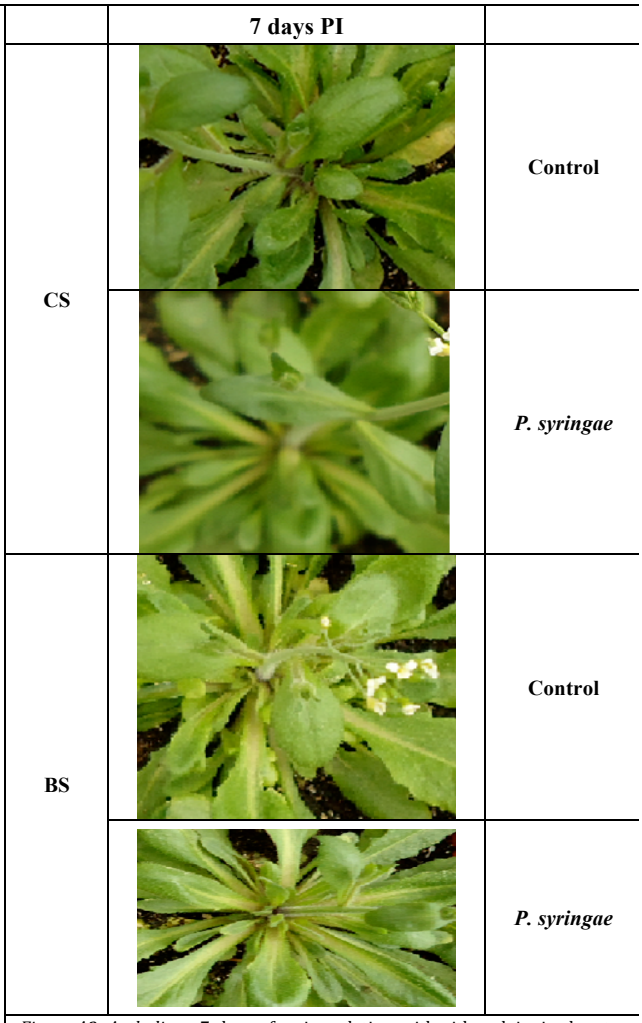

Figure A2: A. thaliana 7 days after inoculation with either deionized water or *P. syringae.* There are no significant differences between soils in regards to pathogenicity.

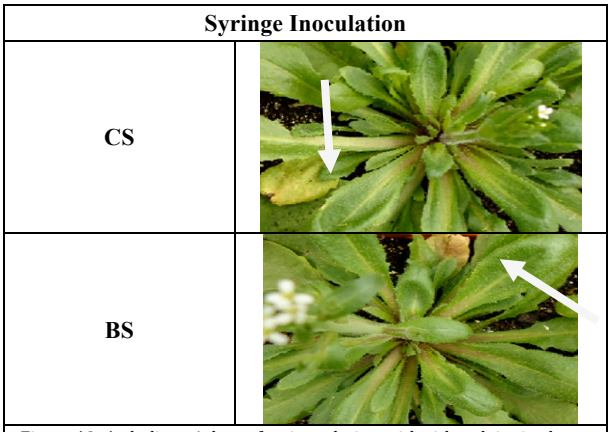

Figure A3: A. thaliana 4 days after inoculation with either deionized water or *P. syringae* via syringe. There is a significant difference between the leaves that were inoculated with *P. syringae* versus deionized water. The white arrows point to the leaves which were inoculated with the pathogen.

Supplement B: Stem lengths for each plant in different conditions. Trial 1 was measured at 4 days PI and trial 2 was measured 19 days PI.

Dip Inoculation

Soil	Trial 1	Trial 2
CS'	13.00	15.10
CS'	9.10	12.50
CS'	8.60	12.20
CS'	N/A	N/A
CS*	10.25	12.50
CS*	9.50	12.10
CS*	9.70	13.20
CS*	7.60	9.00
BS`	N/A	N/A
BS`	13.90	14.30
BS`	4.60	10.00
BS`	10.20	N/A
BS*	11.20	14.10
BS*	13.25	17.00
BS*	10.00	14.50
BS*	12.60	13.10
T-Test		
CS	p>>0.05	p>>0.05
BS	p>>0.05	p>>0.05

`=inoculated

*= no inoculation

Pipette Inoculation

Soil	Trial 1	Trial 2
CS'	4.50	11.70
CS'	3.80	10.50
CS'	1.80	10.00
CS*	6.00	12.10
CS*	6.20	12.00
CS*	7.80	12.10
BS`	5.75	12.00
BS`	2.00	9.50
BS`	5.00	11.50
BS*	2.00	9.00
BS*	4.50	10.20
BS*	4.80	11.00
T-Test		
CS	p>>0.05	p>>0.05
BS	p>>0.05	p>>0.05

`=inoculated

*= no inoculation