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Compositional change of the phyllosphere due to secondary metabolite production

The plant rhizosphere is the culmination of all cellular organisms that are present in the below ground tissues of plants, starting at the base of the above ground plant material, to the end of the root system. These communities are comprised of harmful bacterial (such as pathogenic bacteria like *Agrobacterium tumefaciens*), neutral bacteria, and beneficial symbiotic bacteria like Rhyzobia that form root nodules where they fix nitrogen and transfer it to the host plant in exchange for nutrients (1). While the root microbiome has an extensive list of research focused on the interactions within the rhizosphere, there is lacking research looking at the interaction between the phyllosphere (the microbial community within the above ground plant material) and the rhizosphere even though there is evidence of interaction between both the rhizosphere and phyllosphere (2). The influence of neutral bacteria can also have an affect on the host plants ability to resist pathogens due to an induced systematic resistance (ISR) caused by the presence of said bacterium (2).

One of the prime examples of induced systemic resistance, due to a neutral pathogen, is the effect that *Pseudomonas fluorescens* has on the phyllosphere when it is present in the rhizopshere of the host plant. The presences of *P. fluorescens* within the rhizosphere reduces the ability of *Pseudomonas syringae* to infect the leaves of the plant due to the induced defense response caused by the presence of the chemical 2,4-diacetylphloroglucinol (DAPG) which is released by *P. fluoresces* in the rhizosphere that subsequently spreads throughout the plant (2). The inclusion of this chemical has been found to have significant effects on the growth, and resistance to pathogenic bacteria such as *P. syringae* and fungal infections. This interaction between *P. fluoresces* and its host plant are not the only instance of rhizosphere bacteria and the host plant interacting together, and a converse example is when a bacterium utilizes the chemical galactinol which is released by the host plant as a nutrient.

Previous studies looking at the interactions between the host-plant and the root microbiota have given way to complex interactions where the host-plant (*Arabidopsis thailiana*) releases a defense compound called galactinol which is in-turn is utilized as a nutrient by the bacterial pathogen *A. fabrum*. Galactinol is an understudied defensive compound, and oxidative stress agent, that is present in many species of plants but has not been studied to the extent that other defensive chemicals have been (such as SA or JA). Because of its ability to utilize galactinol as a source of sugar, therefore negating its anti-microbial effects, it allows it to resist the host plant defense response, and instead exploits the defense response for its own gain (3). Therefore, the next question to be asked is if this interaction has any induced systematic resistance for the rest of the plant, and if there are any down stream effects associated with the presence of *A. fabrum* in the rhizosphere.

Specific Aim 1

The first aim of the project will be to investigate if there are any effects of galactinol presence on the growth rate of *P. syringae* *in vitro*. Since galactinol is a defensive compound released by plants in the presence of pathogens, it may have a direct affect on the growth rate if galactinol specifically targets said pathogens – in this case P. syringae. I predict that the presence of galactinol will have a significant effect on the growth rate of *P. syringae* in vitro due to its defensive properties.

- Agar plates will be created using normal levels of nutrients with added galactinol hydrate, and a control group containing nutrients but with no galactinol.

- Every plate will then be exposed to *P. syringae*, and will be incubated to promote the growth of the bacteria.

- After sufficient time for growth, each plate will be analyzed for the the growth rate of the plant and then compared using a chi-squared test.

Specific Aim 2

The next aim of this project will be investigate if plants that are exposed to *A. fabrum* show signs of ISR due to the interactions between the rhizosphere and phyllosphere. It is predicted that the presence of *A. fabrum* in the rhizosphere will have an effect on the phyllosphere due to the induced galactinol response and the ability for *A. fabrum* to utilize the chemical as a nutrient instead of being negatively affected by it. I expect to see a reduction in the infection rate of *P. syringae* because of the induction of galactinol due to *A. fabrum* presence in the rhizosphere.

- Seeds, Columbia Wildtype (Col-0), for plant will be sterilized and grown in sterile magenta boxes on water agar medium containing essential nutrients for the plant.

- The plants will then be subjected to *Agrobacterium fabrum* C58 inoculation via a syringe filled with bacteria injected into the root space of the agar gel.

- After the plant reaches a proper age, it will be inoculated with *P. syringae* and will be phenotyped after 24 hours.

- Leaves will be acquired in order to confirm the presence of galactinol in the plants using an HPLC.

- Presence of *A. fabrum* in the rhizosphere will be qualitatively identified using PCR targeting the C58 locus which is a common locus to use a a proxy for the presence of *A. fabrum* (5).

- The infection rate of *P. syringae* will then be compared between the negative control and experimental groups to determine if the presence of *A. fabrum* in the roots has an effect on the infection of the phylloshpere.

Specific Aim 3

The final aim of the project is to see the effects of *A. fabrum* presence on the growth rate of *P. syringae* on *A. Thailiana* plants grown in soil. Since *A. fabrum* is a rhizosphere dwelling bacteria it is expected that the presence of soil, and the other microbes therein, may have an effect on the ability of *A. fabrum* to induce the galactinol response due to the presence of other microbes in the system. I expect to see a reduction in the infection rate of *P. syringae* because of the induction of galactinol due to *A. fabrum* presence in the rhizosphere.

- Seeds, Columbia Wildtype (Col-0), will be sterilized and grown using sterile autoclaved soil in a growth chamber.

- The plants will then be subjected to *A. fabrum* inoculation immediately after sowing by spraying the soil with a mixture of the *A. fabrum* C58 at high MOI in order to increase infection chance.

- A negative control, where the plant is not inoculated with *A. fabrum*, will be used in order to see the presence of *P. syringae* in a controlled setting given the growth conditions.

- After the plants reach the proper age, they will be inoculated with *P. syringae* at high MOI on the rosette leaves of the plants, and will be phenotypes after a period of 24 hours.

- Leaves will be acquired in order to confirm the presence of galactinol in the plants using an HPLC machine.

- Presence of *A. fabrum* in the rhizosphere will be qualitatively identified using by performing PCR targeting the C58 locus on the root tissue (5).

- Plants found to have galactinol will then be surveyed in order to determine the effect that galactinol has on the infection rate of *P. syringae*.

**References**

1. Lindow, Steven E., and Maria T. Brandl. "Microbiology of the phyllosphere." Applied and environmental microbiology 69, no. 4 (2003): 1875-1883.

2. Bakker, Peter AHM, Corné MJ Pieterse, and L. C. Van Loon. "Induced systemic resistance by fluorescent Pseudomonas spp." Phytopathology 97, no. 2 (2007): 239-243.

3. The plant defence signal galactinol is specifically used as a nutrient by the bacterial pathogen Agrobacterium fabrum. Meyer et al. 2018

4. Morella, Norma M., Annika L. Gomez, Grant Wang, Michelle S. Leung, and Britt Koskella. "The impact of bacteriophages on phyllosphere bacterial abundance and composition." Molecular ecology (2018).

5. Shams, M., L. Vial, D. Chapulliot, X. Nesme, and C. Lavire. "Rapid and accurate species and genomic species identification and exhaustive population diversity assessment of Agrobacterium spp. using recA-based PCR." Systematic and applied microbiology 36, no. 5 (2013): 351-358.

Figure 1.

- A figure showing where the Rhizosphere and the Phyllosphere are located on a plant.

- Arrows will be added to show the effects, such as the release of galactinol, to the areas of the plant it effects (such as the phyllosphere and the rhizosphere.

Figure 2.

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