AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2016 – 2018

TITLE: Suppressing Virulence Factor Production by the Plant Pathogen *Pseudomonas syringae* using Natural Plant-Derived Metabolites

RESEARCH LEADER: Jeff Anderson

COOPERATORS: none

SUMMARY:

Pseudomonas syringae is a bacterium that causes disease on many crops important to Oregon agriculture such as blueberry and pear. *P. syringae* infects host plants by producing dozens of toxins that suppress the plant immune system. One class of these toxins, referred to as "effectors", are directly injected into host plant cells by the bacterium using a syringe-like apparatus called the type III secretion system. In recent work, the PI discovered that specific chemicals released by plants into their immediate environment are signals for *P. syringae* to begin producing its effectors and type III secretion system. While researching these virulence-promoting signals, the PI also found that several other plant-derived metabolites, namely the amino acids β-alanine, leucine and valine, are potent inhibitors of effector production in *P. syringae* at micromolar quantities. These simple compounds are ubiquitous in nature, non-toxic to humans and are unlikely to have non-specific antimicrobial activity. The overall goal of this project is to investigate if these naturally-occurring and non-toxic chemical compounds may be effective at specifically interfering with *P. syringae* infection processes. Results from this work could provide important insights into the underlying mechanisms of host perception by *P. syringae*, and may lead to novel strategies to control infection of crop plants by this pathogen.

OBJECTIVES:

Objective 1) Investigate the molecular mechanisms of how plant-derived metabolites inhibit effector production in *P. syringae*.

Objective 2) Determine if the inhibitory metabolites broadly act against many different *P. syringae* isolates including those found on diseased plants in Oregon.

Objective 3) Investigate if treatment of plants with the inhibitory metabolites can prevent disease caused by *P. syringae.*

PROCEDURES:

Objective 1) Generate a collection of several thousand *P. syringae* DC3000 mutants using Tn5 transposon mutagenesis. Screen this collection for mutants that are not inhibited by β-alanine using a reporter plasmid consisting of an effector promoter sequence fused to a gene encoding a green fluorescent protein (GFP). Identify transposon insertion sites in mutants that are no longer inhibited by β-alanine, as they may disrupt genes that encode for receptors as well as other proteins necessary for the inhibitory effects of this metabolite.

Objective 2) Test a collection of ~25 *P. syringae* isolates that are infectious on a broad range of host plants for effector production in the presence of inhibitory metabolites using the same fluorescent protein reporter system as in Objective 1. This work will involve introducing the $effector_{pro}$:GFP reporter plasmid into each isolate, incubating the plasmid-containing bacteria in conditions that induce effector production in the presence or absence of β-alanine, and measuring the resulting changes in fluorescence as an indirect measure of effector production.

Objective 3) Infect tomato plants with *P. syringae* isolate DC3000 in the presence or absence of inhibitory metabolites. Assess impacts on disease by counting visible speck symptoms on leaves and by serial dilution plating of bacteria from infected tissue. If effective, additional pathogen assays to assess effects of inhibitory metabolites on disease caused by isolates tested in Objective 2 will be pursued.

SIGNIFICANT ACCOMPLISHMENTS TO DATE: To meet the goals of **objective 1**, we developed a high throughput assay to monitor GFP fluorescence in Tn5-mutated *P. syringae* colonies growing on nitrocellulose filters. Developing this method required the optimization of several washing steps to remove residual rich media from the filters, as well as establishing conditions for imaging GFP fluorescence from colonies using a stereomicroscope. Using this assay, we successfully screened 20,000 *P. syringae* colonies for loss of GFP fluorescence in response to bioactive metabolites that induce effector production, and from this screen we identified approximately 200 mutants that show partial or complete loss of response. We are now in the process of identifying the genes altered by transposon insertion in each of these mutants. In the coming year we plan to repeat this high throughput screen to identify mutants that produce effectors in the presence of inhibitory levels of β-alanine as described in objective 1. For **objective 2**, we have successfully introduced the effector expression GFP reporter plasmid into 10 different *P. syringae* strains and plan to do the same with the remaining 15 strains this term as proposed. Once completed, we will use a 384-well microtiter plate assay we developed to monitor GFP levels in these strains in the presence or absence of bioactive metabolites that either induce or repress effector production. This assay will allow us to rapidly assess the effects of inhibitory bioactive metabolites as proposed for objective 2. Experiments outlined in **objective 3** will be initiated in 2017 as proposed.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

Title: Regulation of *Pseudomonas syringae* Virulence by Plant-derived Chemical Signals
Source: National Science Foundation
PI: Jeffrey Anderson, OSU
Duration: 7/15/16-7/15/19
Total Funds: $646,724

FUTURE FUNDING POSSIBILITIES: If we identify *P. syringae* mutants in Objective 1 that are no longer inhibited by the plant-derived metabolites, this could provide immediate insights into the underlying molecular mechanism(s) involved in effector repression. This knowledge could greatly improve the competiveness of future funding requests from federal programs such as USDA AFRI, as one can propose experiments to manipulate the repression mechanism(s) as a means to inhibit the virulence of this pathogen. If experiments in Objective 2 and Objective 3 reveal that the bioactive metabolites broadly inhibit the virulence of many *P. syringae* isolates and can inhibit disease during plant infection, this would suggest a potential role for these chemical compounds in controlling *P. syringae* diseases on many different types of host plants. These results could form the basis for future funding requests to test their efficacy within other agriculturally-important pathosystems.