Screening potential antagonists for fire blight control

Rachel Vannette, University of California Davis
Robert Schaeffer, John Beck, Steven Lindow, with contributions by Caitlin Rering, Kyria Boundy-Mills and Honey Pathak

ABSTRACT

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a destructive disease of apple, pear, and other pome trees, causing an estimated $100 million per year in losses in the US alone. Currently available microbial products for blight control are limited in their effectiveness due to the need for frequent re-application, incompatibility with other products used in IPM (e.g. copper or lime sulfur), or lack of robustness to variable environmental conditions. Here, we examined microbial strains for suppression ability using two complementary assays (in-vitro and in-vivo/in-flower). Both analyses revealed microbial strains effective at *Erwinia* suppression, including both bacterial and fungal strains with good suppression compared to currently used microbial agents. Our IAA analysis revealed that some of the potential antagonist bacteria and fungi produced the plant hormone indole-3-acetic acid, but others produced no detectable IAA, which is associated with russetting. We conclude that natural systems may be a useful resource for novel biocontrol agents effective at *Erwinia* suppression.

OBJECTIVES

We screened previously unexplored microbial strains for effective antagonists to prevent infection by *Erwinia amylovora*. For effective strains, we used chemical analysis to examine russetting potential (i.e. production of plant hormones including IAA). The Vannette lab has a large collection of bacteria and fungi from native and agricultural California flowering plant species and is experienced in conducting microbial competition assays (e.g. Vannette & Fukami 2014, Vannette & Fukami 2016). Our previous work demonstrates that microbial strains isolated from flowers can effectively suppress competitors in flowers and our recent preliminary lab work suggests that many can be effective at suppressing the growth of *E. amylovora* in a floral nectar analog.

For year 2 of our project, we proposed the following objectives:

1) Complete screening of selected bacterial isolates for *Erwinia* suppression using forced flower assays
2) Examine russetting potential across effective isolates chosen from forced flower assays
3) Assemble and test suppression of *Erwinia* by consortia (communities) of antagonists

PROCEDURES

Objective 1.

Cut branches were taken from pear growing at the UC Davis Plant Pathology Orchard (Armstrong) in early spring 2018 and stored in a cold room until bloom was forced. Branches were taken into the growth chamber and upon flower anthesis, individual flowers were inoculated with microbial solutions of potential antagonist isolates (10^7 cells/ml). After one day, flowers were inoculated with ice-nucleating strains of *Erwinia* and incubated for 2 days. Inoculated flowers were subjected to a freezing assay to determine *Erwinia* growth (Mercier and Lindow 2001), where greater *Erwinia* growth increases the temperature at which flowers freeze. We replicated this assay on flowers using 20 potential antagonist strains inoculated individually into flowers using in the forced-flower assay, with each treatment replicated using 20 individual flowers. An additional 20 flowers were used as *Erwinia*-only controls, with an additional 20 serving as no-inoculation controls. This assay has been shown to be predictive of antagonist activity on pear trees (Mercier and Lindow 2001).

Objective 2.

Antagonist strains were grown in artificial media for 3 days at UC Davis. Artificial nectar solution containing 15% sugars comprised of sucrose, glucose and fructose with nonessential amino acids as a nitrogen source. Microbial-conditioned media was freeze-dried and sent to collaborator Beck for chemical analysis for the production of microbial metabolites, including the plant hormone IAA which is often responsible for fruit russetting.

Analysis of IAA was performed as follows: 3-Indoleacetic acid (IAA; 98%) was purchased from MilliporeSigma (St. Louis, MO) and Optima® grade water and acetonitrile were purchased from Fisher Scientific (Hampton, NH). Prior to shipment to Gainesville, FL for analysis, conditioned, cell-free nectar samples (1 mL) were lyophilized to dryness or near dryness (≤ 20 µL) overnight. Samples were shipped on ice and stored frozen (-80 °C). Immediately prior to analysis, samples were re-suspended in 200 µL ultrapure water (>18.2 MΩ·cm), then thoroughly mixed and syringe-filtered (0.22 µm, 13 mm, PVDF, Foxx Life Sciences, Salem, NH) to an insert-equipped autosampler vial for analysis. IAA was quantitated by injection of calibration standards (prepared in ultrapure water and syringe-filtered as nectar samples. Four IAA ions (176.070, 130.064, 198.051, 107.510 *m/z*) were integrated and their peak areas summed and fitted to a linear calibration curve with $1/x^2$ weighting.

Samples were injected (20 µL) on an Agilent 6550 ultra-performance liquid chromatograph coupled to a hybrid quadrupole orthogonal time of flight mass spectrometer (LC-QTOF; Santa Clara, CA, USA) equipped with a dual Agilent Jet Stream electrospray ionization source operated in positive ionization mode. To prevent unnecessary signal deterioration due to the sample matrix, flow from the LC was only diverted to the QTOF source from 1–8 min. IAA was eluted (retention time 6.5 min) with an Aglient Eclipse Plus C18 RRHD analytical column (100 x 2.1

mm i.d., 1.8 µm particle size) and corresponding guard column held at 30 °C and a binary gradient program of 0.1% formic acid in both A: water and B: acetonitrile as follows: initial conditions, 5% B at 0.4 mL min^{-1} flow rate; linear ramp to 100% B in 10 min, flow rate increased to 0.8 mL min^{-1} from 8.5 to 9 min to rapidly flush the column after IAA elution; 12 min total run time; 3.5 min post-time. The QTOF was operated in MS scan mode (70-2000 *m/z*) at 3 spectra s^{-1} with the following conditions: capillary voltage, 3000 V; nozzle voltage, 2000 V; gas temperature, 200 °C; gas flow, 13 L min^{-1}; nebulizer pressure, 20 psig; sheath gas flow, 12 L min^{-1}; octopole RF, 750 V.

Objective 3. We compared EA suppression using freezing assays by subtracting the average proportion of flowers frozen at a given temperature after inoculation by a specific microbial taxa from the freezing temperature of the Erwinia-only controls. Positive values indicate improved suppression (freezing at a lower temperature) whereas negative values indicate improved Erwinia growth (freezing at a higher temperature). Mixed isolate communities of the most promising isolates, including comparisons with single-isolates of PfA506 and *Aureobasidium pullulans* were performed.

Brief summary and status for each objective:

1. We completed screening of all selected bacterial isolates using both in-vitro assays (lab plating) and forced flower assays

2. We have grown in culture all screened isolates from in-vitro lab assays and forced flower assays. Chemical analyses are pending, although delayed due to instrument maintenance and now COVID-19 related shutdowns

3. We have completed screening of mixed consortia suppression of *Erwinia* in forced flower assays. Results suggest that mixed communities generally offer good resistance potential with little evidence of reduced suppression.

DISCUSSION

A subset of potential antagonist strains were effective in reducing population size of Erwinia amylovora measured by freezing temperature in floral trials. Notable agents included the filamentous fungi *Penicillium brevicompatum*, and the bacterium *Neokomagataea thailandica*, which both exhibited similar suppressive ability to the currently used biocontrol agent *Pseudomonas fluorescens* PfA506. Additional nectar-inhabiting bacteria and yeasts from native systems were also effective, including *Metschnikovia reukaufii* and *M. pulcherrima*, as well as the

osmotolerant *Lachancea thermotolerans* were also somewhat effective at suppressing *Erwinia* growth.

However, we caution that the yeasts in the genus *Metschikowia*, including *M. pulcherrima* were among the highest producers of the plant hormone IAA (Fig 2), which is implicated in the process of fruit russetting. This suggests that despite the ability of *Metschnikowia* and other yeasts to reduce *Erwinia* growth in flowers, this genus may be associated with fruit russetting so may not be ideal to pursue in field trials. However, our analysis did not detect any IAA produced by the yeast *Aureobasidium* pullulans, despite this species commonly being associated with russetting in some locations. As a result, we suggest that IAA production by microbial taxa may be context-specific. Further analyses should attempt to replicate in-flower conditions although nectar availability may limit detection of this compound.

Ongoing work in the Vannette lab seeks to link microbial growth kinetics, resource use, phylogenetic relatedness and effects on host plants to predict suppression. Ultimately, we hope that these models will improve biocontrol selection workflows and robustness of suppression under various environmental conditions