A screen of nodule-specific cysteine-rich (NCR) peptides for control of the HLB bacterium in citrus

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Year 1 of 1 (75% Complete)

Objectives

1. Complete the screen of NCR peptides
2. Deliver NCR peptides to CLas infected Asian citrus psyllids via artificial diet and assay impact on CLas transmission

Problem and Significance

The gram-negative bacterium '*Candidatus* Liberibacter asiaticus' (CLas) is implicated as the causal agent of citrus huanglongbing (HLB), a destructive disease of *Citrus* species worldwide. The CLas bacterium is transmitted between citrus trees in the U.S. by the invasive Asian citrus psyllid, *Diaphorina citri*, which spread to the United States in the 1990s, devastating the Florida citrus industry and now threatening citrus growers in California and other citrus growing U.S. states. While a variety of HLB mitigation strategies are under active investigation, antimicrobial peptides are especially promising since they are small, naturally occurring, easily synthesized for laboratory testing, and can be delivered (genetically or via injection) directly into citrus and other crops. The nodule-specific cysteine-rich (NCR) peptides are a family of legume derived antimicrobial peptides with wide-spectrum bactericidal activity, including soil-dwelling bacteria closely related to CLas. However, the utility of NCR peptides as therapeutics to stop HLB disease in citrus trees is unknown. Coupled to novel citrus delivery strategies under development, NCR peptides may provide CA citrus growers a new, effective tool to help combat HLB disease spread.

Benefit to Industry

Citrus growers will have the option to use products based on naturally occurring plant-derived antimicrobial NCR peptides for the control and management of HLB in commercial citrus groves, nurseries and residential areas. NCR peptide products will provide growers with an effective new tool for HLB management. Due to their unique chemistry, NCR peptides are structurally stable antimicrobials that exhibit diverse modes of action. A set of NCR peptides can be combined with other approaches to create an effective resistance management program for HLB control.

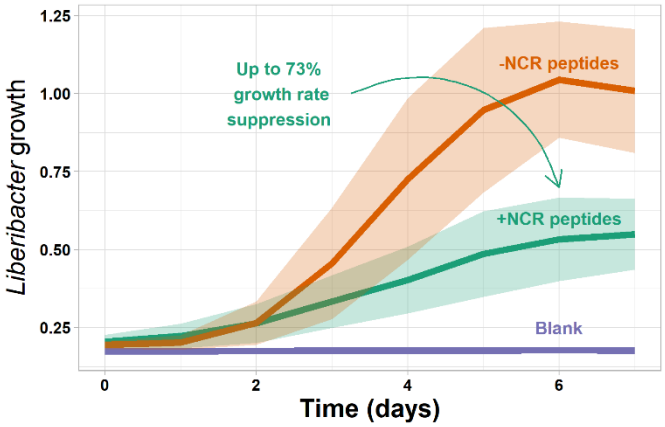

Figure 1 Growth dynamics of Liberibacter crescens *with and without NCR peptides. Y-axis values indicate absorbance units at 600 nanometer (nm) wavelength of L. crescens BT-1 cells in liquid broth.*

Progress Summary

For Objective 1, we have finished an *in vitro* screen of 182 NCR peptides (at 0.1 and 1 milligram (mg)/milliliter (ml) concentration) for antimicrobial activity against the cultivated surrogate of CLas, *Liberibacter crescens* strain BT-1, hereafter *L. crescens*. The screen identified 47 NCR peptides with growth rate inhibition (GRI) of *L. crescens* between 25 and 73% at 1 mg/ml concentration in liquid broth culture (Figure 1). The top 15 candidates (60 – 73% GRI) have been selected for synthesis at a greater scale for tasks related to

Objective 2 (see below) (Figure 2). Currently, we are testing a fractional factorial design to identify significant interactions among combinations of the top ten NCR peptides (which fell in the 60-73% range) with greatest GRI. The number of pairwise combinations to be tested for ten peptides without a fractional factorial design would be 1,024, but this number is reduced to only 128 peptide combinations using a fractional factorial design. Considering the diverse mode of action of NCR peptides, the identification of significant interactions among them may indicate synergistic, yet distinct modes of antimicrobial activity and may limit the development of resistance by CLas. A total of 48 combinations have been tested for activity against *L. crescens*, with the remaining 80 combinations in progress.

For Objective 2, large-scale synthesis of the top 15 performing peptides is in progress. We will begin to test whether the top 15 NCR peptides (individually or in combination) interfere with CLas transmission by the Asian citrus psyllid. Specifically, we will challenge CLas-infected psyllids with artificial diet containing NCR peptides for five days. These psyllids will then be transferred to containers with whole, uninfected citrus leaves. The psyllids will then be allowed to feed on uninfected citrus leaves for 7 days, at which point we will use a molecular diagnostic method to detect CLas in the leaf tissue. We expect to see low or no CLas titer in citrus leaves challenged with psyllids that were exposed to artificial diets with NCR peptides.

We will also examine the effect of NCR peptides on CLas titer in infected citrus trees. We will soak infected citrus leaf petioles in solutions with and without NCR peptides and assess CLas titer over time. Both the artificial diet assay and citrus leaf assay enable a comprehensive, *in vitro* assessment of the effectiveness of NCR peptides to kill CLas in both the psyllid and citrus hosts.

Our team was awarded a new USDA grant which will encompass large-scale tree delivery and field trials of the promising NCR and other antimicrobial compounds discovered in this CRB-funded research to test whether treating whole trees with the NCR peptides provides protection against HLB.

In sum, we identified 15 NCR peptides that significantly decrease the growth rate of the cultured surrogate of CLas, *L. crescens*, compared to controls. We are testing the effectiveness of combinations of NCR peptides on growth inhibition of *L. crescens*. We will apply these peptide combinations in psyllid artificial diet assays to assess their effects on CLas transmission by psyllids.

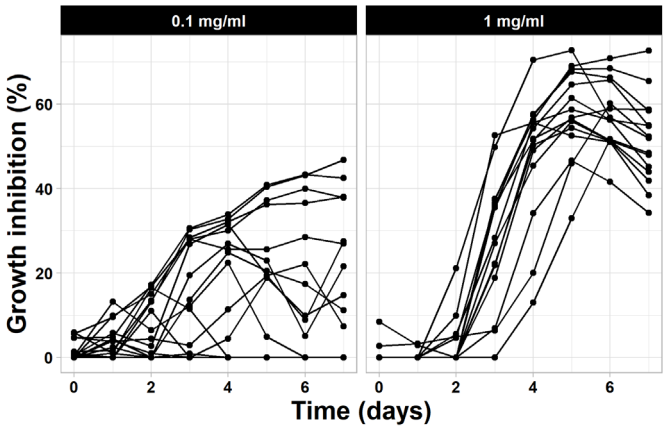

Figure 2 Percent growth inhibition of Liberibacter crescens strain BT-1 in broth culture by the top 15 most inhibitory NCR peptides at two different concentrations. Growth of L. crescens *strain BT-1 cells in liquid broth measured in absorbance units at 600 nm wavelength. Percent inhibition was calculated by subtracting mean absorbance value of NCR peptide treatment from the growth control (no peptide), dividing by the growth control, and multiplying this value by 100.*

CRB Project # 5300-202

Publications and Presentations

Heck et al. 2020. Scientists screen for plant-based antibacterials. *Citrograph* 11(1): 62-63.