CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD March 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Screening and application of antibacterial producing microbes to control citrus Huanglongbing

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/12/2016	Citrus Research and Development Foundation	HLB epidemiology and mitigation of HLB by cultural pra

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Our hypothesis is that application of antibacterial-producing bacteria directly to citrus root could suppress Las population in the roots and control Las. Application of antibacterials in this manner will avoid the strict restrictions of application of antibiotics on crops and ease public concerns since those bacteria are naturally present in the soil and are associated with plant roots. In order to achieve the goal, the following objectives will be conducted: Test antibacterial-producing bacteria against Liberibacter crescens and other Rhizobiaceae bacteria which are closely related to Las. We will mainly test the antagonistic effect of Bacillus, Paenibacillus, Streptomyces and Pseudomonas strains Agrobacterium tumefaciens, Sinorhizobium meliloti, and L. cresens; Control HLB using antibacterial-producing bacteria. For the field test, we will investigate how antibacterial-producing bacteria affect HLB disease severity, Las titres, and citrus yield, survival of the antibacterial-producing bacteria in the rhizosphere and expression of the antibacterial biosynthesis genes in vivo.
We have isolated Streptomyces spp. Bacillus spp. Paenibacillus spp., and Pseudomonas spp. from Florida groves. Multiple isolates showed antimicrobial production activity.
We tested 27 antibacterial compound producing bacteria. These strains had been recovered, purified and confirmed by 16S rDNA sequencing. The antagonistic activity against Agrobacterium, Sionrhizobium meliloti and Xanthomonas citri pv. citri was determined. 5 strains, belonging to Paenibacillus, Burkholderia, Paenibacillus, Streptomyces and Streptomyces showed good antagonistic activity. Three bacteria showing high antimicrobial activities have been sequenced to help us understand the mechanism. Currently, the genome sequencing was finished and we are analyzing the results.
Four bacterial strains: two Burkholderia, one Pseudomonas geniculata, one Rhodococcus strains have been tested for their activity in and all showed induced plant defenses and against infection by Xanthomonas citri.
To further study the antimicrobial producing bacteria, tow Burkholderia strains have been labeled with GFP tag. Seven other strains are being labeled with GFP or RFP tag. We also investigated the antibiotic genes in nine antimicrobial producing bacteria that we isolated previously.
These strains were inoculated to citrus roots and the colonization was determined by inoculation and recover method in lab condition using small citrus seedlings. Around 10E8 cfu were inoculated to each seedling. Approximately 10E4 cfu were recovered from roots 20 days after inoculation (dpi). In a separate experiment, two Burkholderia strains were tested and up to 10E5 cfu/g soil was recovered at five days post inoculation.
For the field trial, we have selected the grove and conduct survey on HLB disease severity. We compared different delivery methods to improve the efficacy of beneficial bacteria. A root drench delivery method has been established. Pseudomonas geniculata strain 95 was applied to trees in Block 9, CREC on Oct 22, 2015. And the bacteria are applied every two months. Three concentrations: 10E6, 10E7, and 10E8 CFU/ml were used and 1 gallon bacterial culture was applied per tree using soil drench method. We are investigating HLB disease index, bacterial survival rate and Las titers. The result demonstrated the bacteria can survive in the rhizosphere soil well, however, it did not show significant higher relative abundance inside roots. The bacteria inoculation did not signficantly affect the bacterial community (UniFrac distance p>0.05).
We recently isolated two novel bacterial isolates inhibiting growth of Sinorhizobium which is closely related to Las. Those two isolates were under characterization. Three field trials are being arranged to test three promising bacteria.

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Project Number 916

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $144,670

Form PR-12 Quarterly Reports **SUBMIT REPORT**

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