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

Rapid identification of antibiotics useful in the control of citrus greening disease

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
03/21/2016	Citrus Research and Development Foundation	Infection Consequences

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

During the period of project 767 funding, we accomplished the following: 1) screened Liberibacter crescens against a wide variety of antimicrobials that we predicted to be phloem mobile (this was done independently of a contract from CRDF to assess compounds of CRDF's choosing); 2) worked with Erik Mirkov of Texas A&M to determine the efficacy of oxytetracycline and streptomycin against tomato yellows disease caused by Ca. L. solanacearum; 3) encouraged and worked with NuFarm to test the efficacy of oxytetracycline in the field against HLB; 4) mutagenesis of L. crescens identified 314 genes that are essential for growth in culture, of those 238 have homologs in Ca. L. asiaticus and can be considered excellent candidates for antimicrobial development; 5) developed a list of seven antimicrobials that are excellent candidates as second generation compounds for treatment of HLB in the field. A paper describing our findings in activities one through three above is in preparation and we hope to submit it soon. A paper on activity four is in revision in Frontiers in Microbiology.

The major findings from these activities are as follows:
1. Three classes of phloem-mobile antibiotics were found to be highly effective against L. crescens: the cephalosporins, the penicillins, and the tetracyclines. The cephalosporins are still used widely in medicine making regulatory approval for citrus use difficult. Penicillins often cause allergic reactions in humans making them difficult to use on a fruit tree crop. The tetracyclines appear to be a very viable option for HLB since oxytetracycline has already been approved for use on bacterial diseases of fruit tree crops.
2. Erik Mirkov of Texas A&M showed that oxytetracycline, but not streptomycin, is very effective against tomato yellows disease. Thus, we recommended that oxytetracycline be tested in the field for control of HLB.
3. NuFarm showed HLB symptom relief in field trials in 2014 and 2015 using oxytetracycline. Streptomycin was not effective in these trials. Since streptomycin is not predicted to be phloem-mobile, we were not surprised by the failure of streptomycin to relieve Liberibacter-induced disease symptoms on tomato and citrus.
4. Thanks to our work on the essential gene list of L. crescens, we now have 238 excellent targets in L. asiaticus for antimicrobial action. This list of genes will soon be in the public domain as a paper describing this work is expected to be published soon in the open access journal, Frontiers in Microbiology.
5. Based on all of the above and on the properties of many antimicrobials tested against L. crescens, we have proposed that a second generation of antimicrobials be tested in preparation for their use in HLB control. These include sulbactim and thiamphenicol and other compounds that require the involvement of other parties. We expect all of them to be effective but have varying degrees of regulatory hurdles. All need to be tested in the tomato yellows assay followed by testing in the field on infected citrus trees. Our view is that a second generation of antimicrobials needs to be available: a) to be used in rotation with oxytetracycline and streptomycin to slow the appearance of resistance to these compounds and b) to replace oxytetracycline and/or streptomycin when resistance dominates in the L. asiaticus population.

PI First Name Eric

PI Last Name Triplett

Email ewt@ufl.edu

Phone 352-392-5430

Organization University of Florida, IFAS

Project Number 767

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

Total Funds (current year)

Form PR-12 Quarterly Reports SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.