CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Las-specific peptides inhibit cell growth in vitro

Proposal Title

Dissecting The Disease Complex of Citrus Huanglongbing in Florida

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/15/11	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

REPORT UPDATE (500 words; summarize your accomplishments)

The objective of this project is 1) to complete the Las genome sequence and conduct comparative genomics studies on the Liberibacter species; 2) to explore the potential role of the microbial community and genetic diversity of Las bacteria in HLB development; 3) to confirm if Las bacteria are seed-transmissible and their role in HLB development.

A complete circular genome of Candidatus Liberibacter asiaticus (Las) was obtained using a metagenomics approach and published in MPMI 22:1011-1020, 2009. In collaboration with Dr. Hong Lin at the USDA-ARS in Parlier, California, we have obtained and published a complete genome sequence of Ca. L. psyllaurous in PLoS ONE 6(4): e19135. doi:10.1371/journal.pone.0019135. All BAC clones of Las were sequenced, and sequence analyses revealed genetic diversity and a potential mechanism of genome reduction.

We have characterized the ATP translocase from Las and proved its function using a heterologous E. coli system. This data was published in J. Bacteriol. 192:834-840, 2010. We are currently developing an antibody-based "drug" to target this protein, aimed at disrupting ATP import, which may be important for its survival. Three peptides screened from a phage display library showed inhibition to the cell growth of Las bacteria in vitro. We have also characterized the individual genes of two putative zinc operons in Las, confirming only one operon responsible for zinc uptake. Each gene of the ZnuABC operon has been characterized, and their expression associated with Zinc metabolism is under investigation.

Seed transmission of Las was tested in grapefruit, sweet orange, sour orange and trifoliate orange. Relatively high titers of Las were detected from both seed coats and inner seed coats collected from HLB-affected citrus plants. A very low titer of Las was detected from the embryos and seedlings using nested PCR and real-time PCR. Most, if not all the seedlings did not show typical HLB symptoms and contained a relatively low Las bacterial titer for HLB, even in the three to four year old seedlings. The results indicated that the seed-transmitted Las could not cause typical HLB disease by themselves, which suggested "Detection of Candidatus Liberibacter asiaticus was NOT necessarily equal to the presence of "HLB disease" in plants." Psyllid transmission study on the Las-positive seedlings was performed. High percentage of psyllids acquired Las bacterium but did not have the same bacterial levels as those from HLB-affected citrus plants. However, it is FIRST TIME that THREE SEED-TRANSMITTED HLB SEEDLINGS were confirmed by PCR using several Las-specific primer sets. Graft transmission of the cutting from this HLB plant confirmed these seed-transmitted HLB. Efforts to reveal the difference between seed-transmitted and field infected Las populations are underway.

Progress on culture of Las bacterium in vitro has been made. Up to 1,000,000 to 100,000,00 cells/ml were obtained within 24 hrs based on qPCR estimation. The Las cells number in the cultures were staggering and decline when they reached to Ct value 21.00 to 22.00 (16S rDNA-based RT-PCR) in liquid media. We are looking into factors affecting further growth.

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Contract Number Duan-162

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

Total Funds (current year) $320,222.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.