CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2012

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Infection traits and growth of 'Candidatus Liberibacter asiaticus' inside microfluidic chambers

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/9/13	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

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

2012 LAS experiments have been completed and data analysis is being conducted. These experiments were similar in design to 2011 experiments, with the treatments being 1/3 King's B (K) medium and commercial grapefruit juice (G) medium. However, the focus was shifted to analyses of the biofilm-like substance formed at the air-liquid interface of culture flasks. 2011 experiments showed that increased formation of this substance was found in juice-containing media, which correlated with prolonged LAS viability. In 2012 experiments, slides with the biofilm-like substance formed on them were stained with Calcofluor (stains exopolysaccharides white) and LIVE/DEAD BacLight (stains live cells green and dead cells red). Micrographs of stained slides showed that exopolysaccharides were present, indicating that the substance was a true biofilm. Results also showed that the majority of cells in biofilm from medium K were dead after several weeks, while the majority of cells were alive in medium G. Analyses are currently underway to determine the percentages of viable and nonviable LAS cells in this biofilm, as well as determine the presence of other potential "helper" bacteria.

Analysis of Illumina MiSeq high-throughput sequencing data of 16S amplicons from experimental samples will soon be completed. Data from the initial citrus-seed inoculum showed that LAS was the largest bacterial component of the inoculum, but significant populations of several other bacterial endophyte species were also present in the seed coats. These other populations, if they also grow in the cultures, could have effects (either positive or negative) on the culture of LAS. The 16S amplicon data from biofilm samples will soon be analyzed. This will indicate if any of these other endophytes were able to grow in the LAS cultures.

A draft of the publication for these data has been written. When the rest of the 2011 and 2012 biofilm bacterial community data is analyzed (qPCR and Illumina data) it will be added to the publication. The data will be presented at the 3rd International Conference on Huanglongbing to obtain any additional insights/comments from other researchers in the field. Subsequently, the publication should be ready for submission.

PI First Name Leonardo

PI Last Name De La Fuente

Email lzd0005@auburn.edu

Phone 334-844-2582

Organization Auburn University

Project Number 307

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

Total Funds (current year) $45,534.40

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.