Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*	HLB Pathology	1/15/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Determining the Microbiome of Healthy and Infected Citrus Phloem Tissue, & Cultivation of Ca. Liberibacter

TITLE and CONTACT INFORMATION

Proposal Title Determining the Microbiome of Healthy and Infected Citrus Phloem Tissue, & Cultivation of Ca. Liberibacter

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Phone 302-224-3450 X305	*Project Duration (years)* 1 *Year of Project* 1
Organization Athena Biotechnologies	*Total Direct Funds (current year)* 346201

REPORT UPDATE (650 words; provide details about your headline)

The two primary objectives of the current proposal are: (1) determine and compare the metagenomes of healthy and infected citrus phloem, and (2) continue to develop and apply AthenaBio's cultivation technologies towards producing a pure culture of Ca. Liberibacter.

Objective 1 Discussion. Laser cutting microdissection (LCM) will be developed to purify phloem-containing sieve cells from both healthy and infected citrus samples. DNA extracted from the purified cells will be analyzed by 16S rRNA sequencing to evaluate the contained microbial diversity. The most diverse DNA samples will be selected for large-scale metagenomic analysis. All DNA extraction and sequencing work will be performed by Dr. Ravel's group (U. of Maryland)

Objective 2 Discussion. AthenaBio will continue to develop and apply its cultivation technologies to isolate a pure cultures of Ca. L. species. In addition to Ca. Liberibacter species, we aim to cultivate other phloem endophytes to provide reference genomes for metagenomic analysis. Two approaches will be employed to disrupt quorum sensing (QS) and thereby enhance growth: (1) physical methods (i.e. a "flow cell") will be used to wash QS signals away from cells, and (2) enzymes will be used to inactivate QS signals through chemical modification. The advantage of the flow cell approach is that knowledge of the chemical structure of the QS signal is not required, and this approach is therefore expected to work on a broad variety of QS systems. Both approaches (flow cell and enzyme) will be combined with various media formulations and incubation conditions.

Work has continued in method development for LCM of citrus phloem cells. Healthy leaf tissue was dissected into small pieces (~5mm), immediately placed in tissue freezing medium and placed on dry ice. Various thickness levels and staining procedures were tested. We believe we have a working method but the instrument was out of order for a period of time. The instrument is now fully functional and we are anticipating DNA analysis to begin by mid-February.