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	7/15/10

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

In vitro culture of LAS : improvement of the primocultures helped with insect feeder cells

TITLE and CONTACT INFORMATION

Proposal Title In vitro culture of the fastidious bacteria Candidatus Liberibacter asiaticus associated with Citrus Greening (Huanglongbing or HLB) Disease.

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Phone (33)4 67 59 39 22	*Project Duration (years)* 2 *Year of Project* 2
Organization CIRAD, Montpellier, France	*Total Direct Funds (current year)* $95,313.00

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

The goal of this project is to obtain the in vitro culture of the bacteria - Ca. Liberibacter asiaticus (LAS)- associated with the Citrus greening syndrome. The strategy consists in getting primo-cultures of the bacteria in insect cells cultures used as feeder cells.

We fulfilled objectives 1 by setting up conditions of LAS inoculation in insect cell cultures.

Objective 2 consisted in obtaining primo-cultures of LAS. Among various insect cell cultures in various culture media tested, two drosophila cell lines (D-mel2, DS2) and a mosquito cell line (C6/36) were selected. Those cell lines show a detection of LAS after at least 3 transfers, the minimum number of transfers after which we consider them as primo-cultures.

Detection: LAS presence is detected by direct PCR. We designed new primers to avoid non specific amplification that occurred with A2/J5 (LAS β-ribosomal protein operon) and OI1/OI2c (LAS 16S rDNA), commonly used LAS specific primers. Those non-specific detections tended to occur when working with high insect cell density or when a close bacterium was present (ie: Bradyrhizobium). We had similar difficulties to quantify LAS in insect cell cultures with previously described qPCR conditions. LAS bacteria could be detected by qPCR but using the primers HLBr/HLBas/HLB probe lead to non specific signals in our cell culture conditions. New primers were designed, giving more reproductible and reliable LAS detections in our cell cultures. We are currently adjusting qPCR conditions to our material. We periodically sequence the PCR fragments obtained to validate the presence of LAS.

For objective 2, We continue to test new insect cell lines. The Keyhani's lab recently sent us Diaphorina citri cell lines. As soon as the lines are maintained and stable, we will test our inoculation protocol on D. citri cells to obtain LAS/ D. citri co-cultures.

Our objectives 3 and 4 are aiming to improve culture conditions to get a higher bacterial titer and to clear the insect cells from the primo-cultures (axenization).

Objective 3: Axenization. Some of our LAS/Aedes or Drosophila primo-cultures were obtained with high insect cell concentrations. In order to axenize those primo-cultures, we tried to progressively dilute the concentration of insect cells through each new passage. However, we lost LAS detection due to faster growth rate of the insect cells, meaning by diluting the insect cells, we diluted even more the bacteria cells.

We are currently testing new culture medium and conditions to contain the insect cell growth itself and we are analyzing new LAS inoculations in these cell cultures.

Objective 4: Medium optimization. To maintain the bacteria for a longer period of time, over more transfers and to reach higher bacterial concentrations, we started complementing the primo-cultures with various sugars, vitamins described in citrus/periwinkle phloem. In parallel we analyzed metabolic pathways potentially encoded by the released Liberibacter genome sequences to define limiting factors and/or growth inhibitors. We are monitoring several LAS positive co-cultures with different additive to improve LAS concentration and decrease the insect cell ratio. Some of these cultures are positive after more than 10 transfers are we are continuously refining our culture media with new additives.