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	1/20/10

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

In vitro culture of LAS in association with insect feeder cells. Primo-culture and improved cultivation.

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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Organization CIRAD, Campus International de Baillarguet	*Total Direct Funds (current year)* $95,313.00

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

We want to obtain an in vitro culture of Candidatus Liberibacter by co-culturing the bacteria with insect cells to study Candidatus Liberibacter physiology, metabolism, virulence and its interactions with the insect vector.
We tested various stable insect cell lines to in vitro culture Candidatus Liberibacter asiaticus (LAS), the Asian form of HLB disease, also found in Florida.
We tested different inoculums (from various citrus and periwinkle plants) on different insect cell lines. We checked for the presence of LAS in inoculated cell cultures by direct PCR.
Current results:
- After Mamestra, Spodoptera, Drosophila cell lines, we tested an Aedes albopictus insect cell line. We didn't detect LAS presence after inoculation. Actually, citrus inoculums have a deleterious effect on Aedes cells and we are now focusing on periwinkles inoculums.
- We detected LAS in two lines of drosophila cell cultures by direct PCR. One line lost the detection after 6 transfers. Signal of detection was confirmed to be LAS. For one line, we are currently reaching the 15th culture transfer with a detection by PCR to be confirmed by sequencing. The detection remains very weak and we need to improve the conditions to get a higher titer.
- The detection of the bacteria by direct PCR peaks at day 7 after transfer and then declines. The bacteria are multiplying and seem to follow the drosophila cells growth. We began to set up multiplex qPCR assays. Primer sets suitable for qPCR and specific of LAS and drosophila detection were selected and tested with success.
- In order to reach higher bacterial concentrations, we tested complements of the culture medium: various sugars, vitamins or trace elements. We analyzed metabolic pathways potentially encoded by the released Liberibacter genome sequences to improve growth conditions and to define limiting factors and/or growth inhibitors. Of the complements tested some had a positive effect on the detection of LAS but negative effects on insect cells survival. With the decline of the insect cell cultures the bacteria was no longer detected. Currently, sodium pyruvate and sterile citrus juice had a positive effect on LAS detection but a lethal effect on insect cells. Proline and fructose had a positive effect on the bacteria detection and are now added to our co-cultures.
- We analyzed insect cell culture medium sugar and amino-acids variations over culture time to identify potential LAS growth limiting factors. Rapid depletion of glucose and of some amino-acids by drosophila cells could be limiting factors and are candidates for our medium improvement.
Current work:
- We are now setting up multiplex qPCR assays to monitor the ratio of LAS and drosophila DNA over culture time.
- We are combining complements to the insect cell culture media and looking for new ones to improve LAS concentration
- We are analyzing insect cell culture medium minerals and trace elements variations over culture time to identify potential LAS growth limiting or improving factors.
- We are testing new insect cell lines to get more consistent presence of the bacteria and higher concentrations.
We are reaching our milestones for the first year of this project and we will look into the axenization of our primo-cultures.