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	Management	02/19/2010

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

One step nested PCR and external membrane-based peptide synthesis under development

TITLE and CONTACT INFORMATION

Proposal Title Diagnosis of Candidatus Liberibacter asiaticus in plant and vector based on molecular and serological approaches

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Organization Centro de Citricultura	*Total Direct Funds (current year)* 32500.00

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

As previously reported the optimization the one step Nested PCR for diagnosis of Ca. Liberibacter asiaticus are under development. The most difficult step nowadays is to obtain for the one-step Nested PCR the same sensitive already worked for the double-step Nested.

Concerning the serological approaches we selected nine different targets which were sold for peptides synthesis. All of these target genes were based on Ca. Liberibacter asiaticus genome sequences and were related to membrane structure according to annotation from NCBI database. The translation of genes sequences to amino acids were done using the Translation Tools (http://ca.expasy.org/tolls/dna.htm) and the hydrophobic and antigenic characteristics were checked by the Antigenicity plot (http://www.bioinformatics.org/JaMBW/3/1/7/). Potential regions based on hydrophobic and antigenic characteristics are shown in the graphics format (data not shown). We used the PSORTdb (http://db.psort.org/) software for localization of selected proteins in the genome. Information about the sequences of peptides and others are bellow show.

General peptides information.

Peptide name	Product	Reference sequence NCBI	Peptide sequence
20-30	Outer membrane protein	YP_003065011.1	SAQAADPVRRA
135-150	Outer membrane protein	YP_003065011.1	YATVGPDVAQKYETGK
118-130	Outer membrane lipoprotein omp19	YP_003065458.1	WNIIDEDSFELKN
133-146	OmpA/motB	YP_003064960.1	PSVYGHDEDAYAKR
28-38	possible lolA type protein	YP_003065211.1	YPITKDQSVKK
164-190x	possible lolA type protein	YP_003065211.1	NWKIMDSSRR
53-79	outer membrane lipoprotein	YP_003064612.1	ILSYFQKDGKFKTISTDGS
130-149 x	outer membrane lipoprotein	YP_003064612.1	LTELPSSKPQDDVAEIPEDP
35-50	OmpA/MotB	YP_003065366.1	KVFLHKSNDTDIVNKRFGSSLDKAED

Next steps

The next steps for this project will be focused on the optimization of one-step Nested PCR for diagnosis of Clas as well as the antibody production that will be started as soon possible we had received the peptides that were sold for synthesis.