# 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 | 7/24/10 |

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

Recombinant antibodies against the HLB pathogen are in development by team of scientists

**TITLE and CONTACT INFORMATION**

*Proposal Title* PREPARATION OF ANTIBODIES AGAINST CANDIDATUS LIBERIBACTER ASIATICUS

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| *Phone* 301-504-6374 | *Project Duration (years)* 3 *Year of Project* 1 |
| *Organization* USDA ARS | *Total Direct Funds (current year)* $221,900 |

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

A scFv library with activity against 'Ca. Liberibacter asiaticus' has been prepared at Beltsville. mRNA was purified from mouse spleens and converted into cDNA. The mice had been immunized with psyllid extracts confirmed to be carrying a high concentration of "Ca. Liberibacter asiaticus" A complete library of variable heavy chain (VH) and variable light chain (VL) genes were made by PCR amplification of the cDNA using a set of 44 primers. The (VH) and (VL) gene segments were then joined in a random combinatorial fashion by overlap extension PCR. The scFv genes were then ligated into the pKM19 phagemid vector which was used to infect Escherichia coli DH5α F' cells with the aide of a helper phage. The resulting phage library is presently being screened to select phage clones expressing antibodies that bind to "Ca. Liberibacter asiaticus". Our first attempts to select desired antibodies using extracts from HLB-infected rough lemon were not successful, probably because the concentration of the target bacteria in the rough lemon extracts was too low. We have therefore modified the screening procedure by incorporating magnetic microbeads. These microbeads bind to rabbit antibodies. To use them we raised standard polyclonal antisera in a rabbit against the outer membrane protein of "Ca. Liberibacter asiaticus". These beads are added to plant and insect extracts to bind the "Ca. Liberibacter asiaticus" and then concentrated by magnetic separation. The phage libraries are then added to the "Ca. Liberibacter asiaticus" on the beads and screened in that manner. This process is ongoing. We have also cloned genes encoding several proteins with potential direct relevance for the control of HLB. Correct cloning was confirmed by DNA sequencing and these genes have been expressed in E. coli and the encoded proteins are being purified. These proteins will also be used as targets to capture scFv antibodies that bind to them. The proteins cloned thus far for this purpose include a dinucleotide polyphosphate hydolase, a pilus component protein, a type IV pilus component protein, and a polysialic acid capsule expression protein. These proteins will be biotinylated, combined with strepavidin coated magnetic beads and used to bind phage expressing antibodies that recognize these targets. Thus we will finish with uncharacterized scFv antibodies binding "Ca. Liberibacter asiaticus" as well as a set of antibodies that bind to specific pathologically relevant proteins of the pathogen.