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	3/12/10

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

Antibody libraries against the HLB pathogen are in development by scientists at the USDA, University of Florida and Agdia

TITLE and CONTACT INFORMATION

Proposal Title PREPARATION OF ANTIBODIES AGAINST CANDIDATUS LIBERIBACTER ASIATICUS

Principal Investigator John S. Hartung	*PI Last Name* Hartung
Email john.hartung@ars.usda.gov	*FDACS Contract Number* 95
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)

Plants infected with 'Ca. Liberibacter asiaticus' were established and propagated at Lake Alfred, Ft. Detrick and Beltsville. Some of these plants were used to raise large numbers of psyllids to be used to immunize mice. Others were grown and used as plant extracts to screen antibody libraries. Visiting scientists were recruited from Sigma-tau Pharmaceuticals SA (Rome, Italy; Dr. Minenkova, an international authority on scFv libraries) and from Luzhou Medical College, (Luzhou, PRC; Dr. Yuan, performing the scFv work in Beltsville). A large number of psyllids from Ft. Detrick and Lake Alfred have been screened for the presence of 'Ca. Liberibacter asiaticus', and the 3% of the psyllids with the highest titer of bacteria were used to inject BALB/c mice. This required the development of a rapid and efficient method to make psyllid extracts which could be assayed by q-PCR without DNA extraction so that intact bacteria were available for injection into the mice. Each injection contained extracts of 1-2 insects at 2-5 x 108 'Ca. Liberibacter asiaticus' per insect mixed with adjuvant. Two sets of mice were immunized. One set was taken to Agdia after a series of four immunizations and used to create standard monoclonal antibodies using hybridoma technology. The second set of mice received an additional immunization and was used to create a scFv library in the bacteriophage vector pKM19. Promising antibody expressing cell lines have been identified at Agdia. These antibodies appear to react with the outer membrane protein of 'Ca. Liberibacter asiaticus' purified from Escherichia coli containing the OMP gene cloned in an expression vector. These antibodies are being purified and characterized further. While screening these hybridomas we observed cross reactions to phloem extracts from healthy sweet orange fruit. This was not expected since the mice were immunized with psyllid and not plant extracts. The nature of the cross reacting antigen is presently unknown. A scFv library with activity against 'Ca. Liberibacter asiaticus' has also been prepared at Beltsville. mRNA was purified from mouse spleens and converted into cDNA. 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 in the initial stages of screening by 'biopanning'against extracts of rough lemon plants. These extracts are confirmed to be high titer for 'Ca. Liberibacter asiaticus' by q-PCR. We anticipate phagemids encoding scFv with specificity against 'Ca. Liberibacter asiaticus' in the near future. These phagemids should recognize a diversity of epitopes in 'Ca. Liberibacter asiaticus', and will be characterized as they become available.

Some other results should be noted. In the process of assaying the psyllids for the concentration of 'Ca. Liberibacter asiaticus' we obtained a dataset of the concentration of 'Ca. Liberibacter asiaticus' present in 686 psyllids. In separate research this dataset may lead to insights into relations among other commensal bacteria and 'Ca. Liberibacter asiaticus' and in the epidemiology of HLB disease. Also, at the beginning of the project, while plant materials were graft inoculated and insects were multiplying on 'Ca. Liberibacter asiaticus' infected plants, we immunized mice with Xylella fastidiosa strain 9a5c (citrus variegated chlorosis) mixed with psyllid extracts. We did this to become familiar with process of creating the scFv libraries and screening them for phagemids with desired scFv. Interestingly, we have identified phagemid expressing scFv which react strongly in ELISA tests to strain Xylella fastidiosa 9a5c but which do not react at all to strains of Xylella fastidiosa associated with Pierce's disease of grapevine. These scFv fragments have interesting potential for diagnostics.