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	1/15/10

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

antibodies for the HLB pathogen are on the way!

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)

During this reporting period Dr. Olga Minenkova of Sigma Tau pharmaceuticals (Rome, Italy) spent 2 weeks in our laboratory with Dr. Yuan and initiated scFv antibody protocols using advanced phage display technologies. This included transfer of the specialized cloning vector and E. coli strains, preparation of competent cells, purification of mRNA, construction of the cDNA library, cloning, and trouble shooting and validation of experimental protocols. As a proof of concept experiment we used Xylella fastidiosa 9a5c (citrus variegated chlorosis) mixed with psyllids to immunize mice. Psyllids were raised and provided by Drs. Brlansky and Damsteegt. Xylella fastidiosa 9a5c was added to psyllids at ~200,000,000 cells per injection and ground in extraction buffer to mimic subsequent injections with psyllids infected with "Ca. Liberibacter asiaticus" from Florida. Mice were sacrificed and mRNA from spleens were used to create the phage library. The library was screened 3 times against X. fastidiosa 9a5c with excellent results. Several antibodies have been identified that react strongly against X. fastidiosa 9a5c but do not react at all against strains of X. fastidosa that cause Pierce's disease. This is a significant 'bonus' result that will benefit both the citrus and grape industries, and demonstrates that our basic approach for making antibodies against "Ca. Liberibacter asiaticus" will be successful. Additional screening will be done by Dr. Nandlal Choudhary in Dr. Brlansky's lab in Florida using ELISA, western blots, tissue blots and immunogold labeling.

While the phage libraries against X. fastidiosa were being screened, mice were injected 3 times over a period of three weeks with psyllid extracts containing "Ca. Liberibacter asiaticus" at ~ 200,000,000 cells per insect. The mice will receive 1-2 more injections and will then be sacrificed for production of both scFv (Beltsville) and monoclonal (Agdia) antibodies. In order to prepare injections of mice, more than 500 psyllids have been individually ground in buffer and sampled by Q-PCR to quantify the amount of "Ca. Liberibacter asiaticus" present in each insect. This is necessary to identify the insects with the highest titer of pathogen (~ top 2% of insects) so that each injection contains enough Ca. Liberibacter asiaticus to induce an antibody response. Symptomatic plants or fruits with the highest titers will be used to screen the phage display library for antibodies against Ca. Liberibacter asiaticus". HLB plants in our greenhouse were individually assayed for titer of "Ca. Liberibacter asiaticus" to be used in the screening of the phage display libraries. HLB symptomatic fruit have been provided by Dr. Brlansky and will be assayed individually to identify sources of extracts to be used in screening the libraries. In related work the outer membrane protein (OMP) of "Ca. Liberibacter asiaticus" has been expressed and purified in the laboratory. This protein has been provided to Cocalico laboratories and they are currently raising polyclonal antibodies against this protein in rabbits. These rabbit antibodies will be useful to capture "Ca. Liberibacter asiaticus" in future experiments that will use the mouse scFv or monoclonal antibodies for specific detection and quantification.