CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Antibodies will help diagnosis and control of HLB

Proposal Title

PREPARATION OF ANTIBODIES AGAINST CANDIDATUS LIBERIBACTER ASIATICUS

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
6/30/12	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words; summarize your accomplishments)

In previous reports we have described the preparation of a scFv library prepared in phagemid vector pKM19. The basic scFv library contains 2 x 10_7th unique phage that bind to different antigens present in 'Ca. Liberibacter asiaticus' (CaLas) and the psyllid vector. We have also reported that we have isolated scFv from this library that bind to epitopes contained in proteins of CaLas that are likely to be related to host pathogen interactions and virulence. These epitopes are found on two flagellar proteins, the major outer membrane protein, a pilus protein, a protein believed to polymerize the capsular polysaccharide surface layer of the bacterium, the TolC protein required for survival in a plant host, and InvA, the invasiveness protein that prevents an infected cell from undergoing programmed cell death by apoptosis. The vector and expression system that we have used for this project allows selection of the scFv antibody expressed from a phagemid genome but packaged on the surface of an M13 particle. This facilitates selection, but large scale expression of the scFv requires cloning of the scFv gene into a cognate plasmid expression vector. We have had problems with many of our scFv at this step, because 'stop' codons can accumulate in the phagemid without affecting the selection process, but prevent expression from the plasmid vector. During this reporting period we have repaired the improper 'stop' codons for several of our scFv to allow full expression of the scFv. These scFv were selected because they showed the desired specificity when selected in the phagemid format, but failed to produce scFv when used in the expression vector. The repairs were done by sequencing the defective scFv and identifying the stop codons, designing primers for a series of PCR that allowed amplification of the scFv while replacing the incorrect codons with corrected sequence, and transforming the corrected plasmid with the scFv into E. coli for expression. We have corrected the sequences for scFv that bind FlhA (scFv B947, B1096, B1072); KpsA (B520, B1199, B1202); the major outer membrane protein OmpA (B743); Pilus protein (B556, B557). These scFv are now available in pKM16, our expression vector for testing. The next steps will require expression of the scFv, purification of the scFv and testing it for yield and specificity against purified antigens. These scFv will be added to our inventory of multiple scFv for each target of interest to improve our chances of finding scFv that will be extremely useful for detection assays and for labeling cells for scientific studies. In the current reporting period a great deal of effort was directed at negotiating with Sigma Tau Pharmaceutical of Rome Italy in an attempt to establish parameters for commercial development of the single chain antibodies developed by this project. Sigma Tau owns the vector used to isolate these scFv and has an ownership interest in the scFv. Mutually agreeable terms for commercialization of the scFv were not found. However arrangements were agreed to enable continued research with the scFv.

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Organization USDA ARS

Contract Number 95

Project Duration (years) 3 *Year of Project* 3

Total Funds (current year) $221,900.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.