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)

Screening of library for antibodies is underway

Proposal Title

PREPARATION OF ANTIBODIES AGAINST CANDIDATUS LIBERIBACTER ASIATICUS

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/19/10	Citrus Research and Development Foundation	Infection Consequences

REPORT UPDATE (500 words; summarize your accomplishments)

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. The outer membrane protein and antibody system for immunocapture of 'Ca. Liberibacter asiaticus' has however not proven to be successful. The polyclonal antibodies made in rabbits against purified outer membrane protein from 'Ca. Liberibacter asiaticus' work very well in detecting the purified outer membrane protein but are not effective in immunocapture. The most likely explanation is that the outer membrane protein is not well enough exposed on the surface of the 'Ca. Liberibacter asiaticus' cell. We have therefore cloned genes encoding the type IV pilus protein of 'Ca. Liberibacter asiaticus', since this protein should be well exposed on the cell surface and should be useful for immunocapture in place of the outer membrane protein. Correct cloning was confirmed by DNA sequencing and these genes have been expressed in E. coli and the encoded proteins and have beenpurified. Emphasis is on recovering the proteins in native, soluble form, which is difficult, but we have now been able to accomplish it. These proteins will be used as antigen to prepare polyclonal rabbit antiserum for the immunocapture portion of the antibody screening process. In related research, we have also cloned and expressed a dinucleotide polyphosphate hydolase, and a polysialic acid capsule expression protein. These proteins, as well as the type IV pilus protein 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.

PI First Name John

PI Last Name HARTUNG

Email john.hartung@ars.usda.gov

Phone 301-504-6374

Organization USDA ARS MPPL

Contract Number 95

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $221,900.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.