CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Visualization and detection of proteins produced by 'Ca. Liberibacter asiaticus' in infected sweet orange plants and vector psyllids

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/6/14	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

We have made progress with the scFv library made with the earlier grant from CRDF. We had previously used the scFv when expressed as part of the M13 phage vector particle in ELISA and dot blot formats. Our efforts in the past quarter have built on that work, and now we are using the scFv alone in tissue print assays of citrus plants to detect 'Ca. Liberibacter asiaticus'. scFv are expressed and purified from from E. coli cells using a 6X His tag incorporated in the scFv protein. Study of the literature showed that the media used to grow the E. coli and other details of the culture conditions greatly influence the yield of scFv obtained from culture lysates. We have found that a very rich and buffered medium, (2X yeast extract Tryptone broth with phosphate buffer) works best. The medium is supplemented with glycine, sucrose and IPTG at various stages of the expression protocol. With this protocol we have produced purified scFv at concentrations in the mg/ml range. Differences are observed among different scFv clones. Results from SDS-PAGE gels are consistent with post translational folding being problematic for some scFv as has been reported in the literature.

The tissue print assays continue on nitrocellulose membranes. Cross reactions with healthy plant tissue can be a problem, especially if the concentration of scFv is too high. However, color development is observed in the vascular cylinder (phloem) of HLB infected petioles but not in comparable petioles from healthy trees. In some tissue prints, color development is observed in discrete spots outside of the phloem cylinder. Similar results are obtained with all scFv that were selected to bind to proteins expressed on the surface of 'Ca. Liberibacter asiaticus'. These targets include an ATPase associated with the type IV pilus, a pilus assembly protein, two flagellar proteins, the major outer membrane protein OmpA, and the efflux protein TolC.

Previous work has used a secondary monoclonal antibody directed at the 6X His Tag of the scFv molecules conjugated with alkaline phosphatase and blocking of the membranes with skim milk. We have recently made tissue prints with a modified technique, using 'Super block', a commercial product used in Northern and Southern blotting on nitrocellulose membranes and detection with a monoclonal antibody directed at the FLAG epitope on the scFv. This protocol produces remarkably sharper tissue prints with dramatically reduced background, and color tightly focused as a ring in the phloem cylinder of HLB infected, but not healthy petioles.

We have had unexpected results with the negative controls in these assays: When the secondary anti-FLAG monoclonal is used alone, without any scFv, it produces color tightly focused as a ring in the phloem cylinder of HLB but not healthy citrus. Thus the anti-FLAG monoclonal alone detects the presence of disease. As a further control, we have prepared polyclonal antibodies against the major outer membrane protein and detected them with alkaline phosphatase. The polyclonal antibodies produce distinct spots of color corresponding to individual phloem cells infected with 'Ca. Liberibacter asiaticus'. This is a useful assay for 'Ca. Liberibacter asiaticus', and we are using it to describe the distribution of 'Ca. Liberibacter asiaticus' in infected citrus trees, fruit and seed. We have continued work with these antibodies applied to tissue printing of plant samples during the current reporting period. A manuscript on the original scFv antibodies has been prepared and two manuscripts on the tissue printing assays are in preparation.

PI First Name John

PI Last Name Hartung

Email john.hartung@ars.usda.gov

Phone 301-504-6374

Organization USDA ARS MPPL

Project Number 551

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

Total Funds (current year) $114,719.00

Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.