QUARTERLY REPORT for FCATP08: Control of Citrus Greening, Canker \& Emerging Diseases of Citrus

Instructions Complete the fields as requested based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to http://citrus.hivip.org, scroll down to Grants and log in with your user name and password. Scroll down to this project title and click on Submit a Report. Update your profile information if needed, then upload this report as directed.

## 2008-2009 REPORT

Sept. 30, 2008
$\square$ April 15, 2009 $\square$ July 15, 2009

PROJECT CATEGORY (check one)
OPlant Improvement Pathology $\bigcirc$ Entomology Management/Physiology Other

## TITLE and CONTACT INFORMATION

Project Title Culturing Candidatus Liberibacter asiaticus in vitro and verification if the bacterium is the causal agent of citrus huanglongbing

| Principal Investigator Y. -P Duan | Today's Date 01/05/09 |  |
| :--- | :--- | :--- | :--- |
| Email yongping.duan@ars.usda.gov | FDACS Contract Number 13503 |  |
| Phone 772-462-5840 | Project Duration (years) 3 |  |
| Organization USDA-ARS | Total Direct Funds (current year) | \$94,234.00 |

## REPORT UPDATE ( 650 words)

The causal agent of HLB in Florida is believed to be Candidatus Liberibacter asiaticus (Las). Fulfilling Koch's postulates, by isolating Las in pure culture, inoculating it back in to citrus and seeing the same HLB disease, is necessary to conclusively state that Las is the causal agent of HLB. The ability to propagate HLB on a large scale would have a worldwide impact : leading to better diagnostics, more HLB research tools, and increased understanding of HLB replication and infection.

We continue to assess the requirements for the growth of Las in culture. This includes amino acids, vitamins, other supplements, and "helper" organisms that may be necessary to establish successful culture conditions. To date media recipes have been based on those successfully used to isolate xylem-limited or phloem-limited bacteria. We are currently evaluating variants of BBM used for co-culturing Las with Actinobacteria (Davis et al., 2008). While we have been able to isolate Las PCR-positive colonies, an indication of week multiplication of the bacterium, we have not been able to passage these colonies by solid or liquid media. New information from our genome sequencing project (\#13644) is being applied to identify metabolic pathway deficiencies and other possible growth requirements. Initial findings indicate that the enzymes involved in the synthesis of the amino acids tryptophane, tyrosine, leucine, isoleucine, and valine from metabolic intermediates are absent. We are therefore using these amino acids, at varying levels, as supplements to our synthetic media. We are also still persuing a secondary bacteria that may act as a "helper" organism whose presence or metabolic product(s) may be necessary for Las to grow in vitro. A few species of bacteria (Aerations altamirensis, Phycibacter jejuensis and Agrococcus versicolor) isolated from HLB-infected periwinkle appeared to support the growth of Las bacterium in our previous work. We are currently testing P. jejuensis as a helper in our new synthetic media.

We are also using diffusion chamber techniques to simulate the hosts natural environment. We are currently testing new diffusion chamber technology in HLB-affected citrus using a basic BHI medium and 0.03 u pore filter. We are attempting to adapt it for use with periwinkle as well. This may allow us to isolate the organism prior to determining exact growth requirements.

We are also testing inoculation methods in order to fulfil Koch's postulates. Our newly developed protocols for isolating the bacterium from plant tissue and detecting it in half of a psyllid have given us techniques to obtain inoculums and we are currently assessing different inoculation protocols to determine which will be most likely to succeed.

