Toward a Reliable, Insect Cell Culture-based Technique for Culturing *Candidatus* Liberibacter asiaticus Bacteria

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Take Home Message:

- The goal of this proposal is to identify an ACP cell culture system for in vitro culture of *C*Las.
- Establishment of an in vitro culture system for *C*Las is important for functional genomic analysis of *C*Las, fulfillment of Koch's postulates, characterization of *C*Las interaction with the host plant and insect vector.
- Establishing a culture will facilitate screening of antimicrobial agents for use in combating HLB.

Summary: *Candidatus* Liberibacter asiaticus (*C*Las), is the presumed causative agent of huanglongbing (HLB), which has devastated citrus production in Florida and now threatens all citrus growing regions in the United States, including Texas and California. There is currently no cure or durable remedy to combat citrus greening. While the ability to culture *C*Las in vitro would provide huge benefits for analysis of *C*Las biology and for fulfillment of Koch's postulates to confirm that *C*Las causes citrus greening, attempts to culture *C*Las in the absence of other bacteria have failed. Our long-term goal is to provide a reliable, insect cell culture-based method for culturing of *C*Las bacteria.

The goal of this proposal is to identify an Asian citrus psyllid (ACP) cell culture system for in vitro culture of *C*Las. Our objectives are to test for *C*Las replication in axenic culture using optimized insect cell culture media, assess hemipteran insect cell culture systems for *C*Las replication, and establish cell lines from *C*Las-positive psyllids. Once a *C*Las culture is established, we will test for ACP transmission of cultured *C*Las to healthy citrus, and whether inoculation of healthy citrus results in HLB.

Upon completion of this project, we will have a culture system that serves as an essential research tool for increased understanding of *C*Las *C*Las biology and for effective, rapid screening of antimicrobial agents against *C*Las. Outreach activities will facilitate public understanding and grower adoption of antimicrobial strategies for *C*Las management.

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