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

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Summary: *Candidatus Liberibacter asiaticus* (CLAs), 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 CLAs in vitro would provide huge benefits for analysis of CLAs biology and for fulfillment of Koch's postulates to confirm that CLAs causes citrus greening, attempts to culture CLAs 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 CLAs bacteria.

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

screening of antimicrobial agents against CLAs. Outreach activities will facilitate public understanding and grower adoption of antimicrobial strategies for CLAs management.

Take Home Message:

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

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