

4.8 Cocultivation of *Candidatus Liberibacter Asiaticus* with Actinobacteria from Citrus with Huanglongbing

Davis M. J., Mondal S. N., Chen H.-Q., Rogers M. E., Brlansky R.H.

University of Florida/IFAS, Citrus Research and Education Center, Lake Alfred, Florida

Huanglongbing (HLB), also known as citrus greening disease, is a devastating disease of citrus caused by phloem-limited bacteria that have not been grown in culture. Three species, *Candidatus Liberibacter asiaticus*, *L. africanus*, and *L. americanus*, are known. *Candidatus L. asiaticus* and its insect vector, the psyllid *Diaphorina citri*, have been recently introduced into Florida. We attempted to isolate *Candidatus L. asiaticus* using media formulations developed in response to the growth of another bacterium that appears to be related to the Liberibacters based on 16S rRNA gene identities. Cultures were obtained that were PCR positive for *Candidatus L. asiaticus*. However, transmission electron microscope examination of the culture, PCR using generic primers, and sequencing of the PCR products revealed the presence of other bacteria in the cultures. These were actinobacteria related to *Propionibacterium acnes* based on 16S rRNA identities. The cocultures remained after attempts to purify the cultures by single colony isolation suggesting that the bacteria might be mutually beneficial to each other in culture. The cocultures survived more than 10 weekly passages to fresh medium. PCR using *P. acnes* specific primers indicated that actinobacteria are common inhabitants of citrus and psyllids, whether or not *Candidatus L. asiaticus* is present.