Cultivation and Rapid Detection of the Causal Agent of Huanglongbing Disease of Citrus

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Huanglongbing (HLB), an insect-transmitted disease of citrus, also known as citrus greening, results in a green blotchy mottle on foliage, small, lopsided fruit with tree decline and eventual tree death. The causal organism has been identified as a bacterium, however no organism had been previously cultured. The protocol for identifying disease agents is to follow Koch's postulates: isolate the pathogen from the diseased host and grow the pathogen in culture, use the culture to infect a healthy host plant and observe typical symptoms of the disease, and re-isolate the pathogen from symptomatic tissue and grow in culture.

Current diagnosis of the disease has focused on symptoms and a molecular assay called real-time polymerase chain reaction (RT-PCR). RT-PCR assays have identified the presence of a group of bacteria known as Liberibacter that always occur in association with the disease. Current assays, however, lack specificity between the three species, L. asiaticus (Las), L. americanus (Lam), and L. africanus (Laf).

Our research focuses on culturing the causal agent for completing Koch's postulates and providing DNA for sequencing the entire genome.

We have obtained limited cultivation of Las, Lam, and Laf on artificial media. We inoculated a young lemon tree with pure cultures of Las, and HLB-like symptoms occurred after six months. Orange seedlings inoculated with Las or Lam were also positive for HLB. The bacteria were re-isolated from the infected plants and grown in culture, thus satisfying Koch's postulates under artificial conditions.

Although we can cultivate Las, we have not been able to collect enough DNA to have the genome sequenced. The bacteria have an outer wall that has been difficult to break using standard molecular methods. We are currently testing a high pressure cycling system with various temperatures and chemicals to improve extraction of DNA.

The genetic characterization and rapid detection of Liberibacter is another research focus. Strains of Las were collected from across Asia, Brazil, and Florida. Five small regions of DNA were sequenced to evaluate the relatedness within the species. Strains from Thailand and China were quite different from each other and formed separate groups. The strains from Brazil and Florida were highly similar to each other, and as a group more similar to Chinese strains. We are currently developing a database to allow tracking the movement of HLB and identification of the source of infections.

Possible movement of HLB into California through psyllids poses a real threat. To assist surveys for the presence of infected psyllids and/or trees, we have developed a simple RT-PCR assay that can be completed within several hours. Unlike previous assays, our RT-PCR assay unambiguously identifies the Liberibacter species in a single reaction and does not react with uninfected tissue. Isolations can be done to confirm infection of plant tissue. A method is being developed for the isolation of Liberibacter from psyllids. Eventually, a field-based lateral flow detection device using several specific genetic fragments identified for genetic characterization will be developed in collaboration with LANL.

To the left is a Citrus macrophyllus *ten months after inoculation with a pure culture of* Candidatus *Liberibacter asiaticus. Mottling and yellowing symptoms are evident throughout several leaves. Real-time PCR and culturing indicated the presence of* Candidatus *L. asiaticus. On the right is the healthy control.*