

A Next Generation Sequencing (NGS)-based system for unambiguous detection of HLB pathogen

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Year 4 of 2 (100% Complete)

Objectives

1. Effective enrichment of low titer CLAs DNA from HLB suspected samples
2. Identify a cost-effective protocol for sample sequencing
3. Evaluate and finalize an analytical pipeline for accurate and sensitive detection of CLAs

Problem and Significance

Accurate interpretation of Ct value in PCR detection of huanglongbing (HLB) pathogen, “*Candidatus Liberibacter asiaticus*” (CLAs) is critical in HLB management. Detection of CLAs is based on PCR technology that generates a Ct value representing the amount of CLAs. However, a problem could arise with a high Ct value. Some primer sets may lead to a false positive PCR result due to the presence of related but non-CLAs bacterial strains.

Benefit to Industry

A highly reliable CLAs detection system can help state-wide HLB management efforts in California.

Progress Summary

As of July 2021, Objective 1 was 100% completed. Objective 2 was 95% completed, and Objective 3 was 90% completed. A metagenomic pipeline for unambiguous detection of CLAs has been established and ready for further tests with more Asian citrus psyllid (ACP) and citrus samples.

In August 2021, the pipeline of using NGS technology for unambiguous detection of CLAs in ACP and citrus samples collected from California has been officially published in the journal of

Frontiers in Microbiology. The key discovery was that ACP and citrus could harbor two CLAs-related bacteria, called *Mesorhizobium* and *Bradyrhizobium*. The concentration of these two bacteria varied from sample to sample. In the case of high concentration, the two bacteria could obscure the 16S rRNA gene-based PCR process of CLAs detection, particularly in the absence of CLAs, and give a high Ct value or a false positive result.

Despite the maximum telework status of the United States Department of Agriculture, project research continued in collection of additional ACP and citrus samples from California, Texas, and Florida. Of particular mentioning is that with the help from California Department of Food and Agriculture, a CLAs infected ACP sample was collected from a San Diego location where HLB was recently detected and confirmed. Using the metagenomic pipeline, the draft genome sequence of CLAs San Diego strain has been obtained. The San Diego strain was determined to be a PTG1 strain based on the established prophage-typing group (PTG) system for CLAs classification. This CLAs strain is similar to those found in San Bernardino, Riverside and Anaheim. *Mesorhizobium* sp. and *Bradyrhizobium* sp. were also detected in the San Diego sample. However, the two bacteria were in low concentration.

Conclusions

Using the NGS technology, an analytical pipeline or procedure has been developed to identify the possible non-CLAs cause of high Ct in ACP and citrus samples from southern California.

Two bacteria, *Bradyrhizobium* sp. and *Mesorhizobium* sp. could influence PCR detection of CLAs. However, NGS technology can clearly separate the two bacteria from CLAs. In another word, the NGS-based system can unambiguously detect CLAs. This technology is of particular use for early detection of HLB pathogen in California that commonly encounters high Ct values of PCR tests.

In addition, the implementation of this CRB project has led to significant collaborations with other

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institutions in US (California, Florida, and Texas) and around the world (Brazil, China and Mexico). A manuscript describing the NGS-based system for unambiguous detection of HLB pathogen has been published in a refereed journal.

CRB Project #: 5300-188

Publications and Presentations

1. Huang, J.; Dai, Z.; Zheng, Z.; et al. (2021). Bacteriomic analyses of Asian citrus psyllid and citrus samples infected with “*Candidatus Liberibacter asiaticus*” in Southern California and Huanglongbing Management Implications. *Front. Microbiol.* 12:683481. <https://www.frontiersin.org/articles/10.3389/fmicb.2021.683481/full>
2. Silva, P.A.; Huang, J.; Wulff, N.A.; et al. (2021). Genome Sequence Resource of ‘*Candidatus Liberibacter asiaticus*’ from Brazil. *Plant Disease* 105:199-201. <https://apsjournals.apsnet.org/doi/10.1094/PDIS-05-20-1018-A>

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