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CCNB Project #: Yok-18

Project Title: Improved sensitive detection of the HLB pathogen by new gene primers for real time and droplet digital PCR

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2018 Progress Report

- Completed research and published paper (Selvaraj et al. 2018 on a duplex real-time PCR (qPCR) and a droplet digital PCR (ddPCR) procedures for simultaneous detection of two different gene targets detection of "*Candidatus Liberibacter asiaticus*" (CLas). The two targets were the 16SrRNA and the RNR. This procedure allows dual DNA target detection of the same sample in the same reaction tube for unambiguous detection of low titer CLas saving expensive PCR supplies. Results should be binding without need for confirmation of product sequencing. Last year the CCNB asked if USDA, APHIS, PPQ and CDFA can use or accept these results. I discussed this with Mark Nakhla, Laboratory Director, CPHST, Beltsville, MD and they were happy with our work and were already adapting the use of the RNR primer/probe as a confirming test for CLas.
- Completed research and published paper (Dai et al. 2019) on prophage diversity of CLas strains in California specifically identifying 4 different CLas populations found in southern California dooryard trees. This research further confirmed the RNR primer probe for accurate and sensitive detection of CLas and identified additional gene targets to differentiate California CLas populations. This data helps to evaluate entry pathways of the new CLas populations and can assist other research on the use of prophage to control CLas.
- Research will continue to develop a sensitive isothermal amplification procedure for CLas detection using crude citrus extract without DNA extraction or a thermal cycler. The method employs an isothermal recombinase polymerase amplification (RPA) as an alternative to PCR for point of use diagnosis. The reaction mixture would be loaded into a lateral flow strip chamber with results like a pregnancy strip test. The applicability of this procedure depends on its sensitivity and, thus, titer of the CLas in the sample.

Publications

Selvaraj, V., Maheshwari, Y., Hajeri, S., Chen, J., McCollum, T.G., and Yokomi, R. 2018. Development of a duplex droplet digital PCR assay for absolute quantitative detection of "*Candidatus Liberibacter asiaticus*". PLoS ONE 13(5): e0197184. <https://doi.org/10.1371/journal.pone.0197184>.

Dai, Z., Wu, F., Zheng, Z., Yokomi, R., Kumagai, L., Cai, W., Rascoe, J., Polek, M., Deng, X., and Chen, J. 2019. Prophage diversity of "*Candidatus Liberibacter asiaticus*" strains in California" Phytopathology <https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-06-18-0185-R>