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Whole Genome PCR Sequencing Strategy for ‘Candidatus’ Liberibacter asiaticus: Analyzing Sequence Diversity Among U.S. Isolates

Shatters, R.G., Jr.¹, Morgan, J.K.¹, Jara-Cavieres, A.², Stover, E.¹, Powell, C.A.², Duan, Y.-P.¹, and Moore, G.²

¹USDA, ARS, USHRL, Fort Pierce, FL
²University of Florida, Gainesville, FL

The inability to culture the ‘Candidatus’ Liberibacter asiaticus (CLas) bacterium has greatly hindered research on the etiology of the citrus disease Huanglongbing which is associated with this bacterium. This is especially true with respect to possible links between strain/isolate diversity and disease symptom variations and development. Past genetic marker research indicates that there is considerable CLas isolate diversity even within Florida (a location that has only recognized the presence of the disease since 2005); however, no effort has been made to correlate this diversity with symptom differences. To advance our understanding of CLas geographic spread and strain/isolate diversity, we have developed a whole-genome PCR amplification strategy that can be used in conjunction with next generation genome sequencing to rapidly obtain near whole genome sequence for specific isolates. This method was used to generate genome sequence data (~93% of the total genome with an average ~300x coverage) from numerous isolates within Florida that are known to be different with respect to previously characterized genetic markers, and also to compare genomic sequence with isolates that may induce different symptoms within citrus. Results will be presented that show the diversity among Florida CLas isolates and will also be contextualized within the diversity observed sequence variation among global isolates.