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Repertoire of novel sequence signatures for the detection of Candidatus Liberibacter asiaticus by quantitative real time-PCR

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Huanglongbing (HLB) or citrus greening is a devastating disease of citrus [1]. Circumstantial evidence indicates that HLB is caused by Ca. Liberibacter asiaticus in the United States as well as in Asia [1, 2]. Accurate detection of Las in infected trees and psyllids plays important role in regulation and serves as one important control measurement in citrus producing areas without HLB to prevent it from being endemic [3]. Among the diagnosis tools, quantitative real time-PCR (qRT-PCR) based on selective candidate genes/regions like 16S rDNA, β operon, or nusG-rplK regions have been developed and most widely used [4,5,6,7,8]. Generally those sequences are highly homologous across closely related bacterial species, therefore, prone to be less specific to Las and misdiagnosis. In order to specifically detect Las by qRT-PCR, we exploited the known genome sequence of Las and performed an exhaustive sequence search for all the unique genomic regions. By designing the qRT-PCR primers specific to the identified 34 unique genes, we specifically detected the Las with no cross reactivity to the closely related species e.g. Ca. L. americanus and Ca. L. africanus. The sensitivity of most of our primer sets is comparable or better than 16S rDNA based primers. In conclusion, we have identified and experimentally validated the repertoire of novel sequence signatures that can facilitate the detection of Las from the infected plant by qPCR thereby aid in controlling the disease.

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