Title:
The Chemistry behind DNA Isolation from Orange Juice and Detection of 16S rDNA of Candidatus Liberibacter asiaticus by qPCR

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Abstract:
The current standard to diagnose Huanglongbing (HLB) for citrus trees is to take samples from midribs of leaves, which are rich in phloem tissues, and apply quantitative real-time PCR (qPCR) test to detect 16S rDNA of *Candidatus* Liberibacter asiaticus (*C*Las), the putative causal pathogen. It is extremely difficult to detect *C*Las in orange juice because of the low *C*Las population, high pectin concentration, low pH and possible existence of an inhibitor to DNA amplification. The objective of this research was to improve extraction of DNA from orange juice, and detection of *C*Las by qPCR. Homogenization using a sonicator increased DNA extraction by 86%, and stabilized quantification of 16S rDNA in comparison to mortar and pestle extraction, which showed wide variability of Ct values of 16S rDNA. Orange juice is rich in pectin, which has similar physiochemical features to DNA: soluble in water and precipitates in ethanol/isopropanol solutions. Thus, it is difficult to separate the DNA from pectin. However, DNA was successfully extracted by adding pectinase to hydrolyze the pectin. Without going through an elution column, the amplification of plant and microbial DNA in orange juice samples was inhibited by an unknown compound. Thus application of an elution column successfully eliminated the inhibitor. To eliminate errors caused by different methods of sampling, DNA extraction and qPCR procedures, Ct of a cytochrome oxidase (COX) to represent citrus plant DNA was detected as a reference, and a relative unit, $\Delta Ct_{16S\ rDNA\text{-}COX}$ was introduced to express the relative *C*Las population.

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