

3.9 Quantification of viable *Candidatus Liberibacter asiaticus* in hosts using Quantitative PCR with the aid of ethidium monoazide (EMA)

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Citrus Huanglongbing (HLB) is a devastating disease of citrus known to be associated with a fastidious, phloem-limited Gram-negative, yet to be cultured bacterium in the genus *Candidatus Liberibacter*. In the present study we have developed a method to quantify viable *Candidatus Liberibacter asiaticus* (Las) with the aid of ethidium monoazide (EMA) which can differentiate the live from dead cells. Firstly, calibration curves were developed with the aid of quantitative real-time PCR (QPCR) by using plasmid template consisting of a 703 bp DNA fragment of *rplKAJL-rpoBC* (β -operon) region. Standard equations were then developed to quantify Las genome equivalents in citrus, periwinkle, and psyllid, respectively. To overcome the limitation of real time PCR in discriminating live and dead bacterial cells, EMA was used to inhibit the amplification of DNA from the dead cells of Las in plant samples. By using the standard equations and EMA-PCR methods developed in the study we found that the viable cells range from 17-31% in the citrus and 16-28% in the periwinkle. It was indicated that a minimum bacterial concentration was required for HLB symptom development in studying the population of Las in symptomatic and asymptomatic leaves. This is the first report of the use of EMA-QPCR in distinction between the viable and dead cells of any unculturable bacteria. The EMA-QPCR methodology developed in the present study would provide an accurate assessment of viable HLB pathogen, thus providing a tool to study the epidemiology of disease and act as a crucial component for disease assessment and management.