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Authors

Parker, J. K.
Wisotsky, S. R.
Hilf, M. E.
[et al.](#)

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Increases in ‘*Candidatus Liberibacter asiaticus*’ viability and investigations of biofilm-like structures in citrus juice medium

Parker, J.K.¹, Wisotsky, S.R.¹, Hilf, M.E.², Sims, K.R.², Cobine, P.A.¹, and De La Fuente, L.¹

¹Auburn University, Auburn, AL, USA

²USDA-ARS, Fort Pierce, FL, USA

Huanglongbing disease of citrus, associated with infection by the bacterium ‘*Candidatus Liberibacter asiaticus*’ (LAS), has spread rapidly in the US since 2005. Attempts to culture LAS *in vitro* have not yielded a consistently reproducible culture method; therefore, obtaining knowledge about the infection process is difficult. To determine conditions which sustain LAS viability, LAS inoculum obtained from seeds of fruit from infected pomelo trees (*Citrus grandis* ‘Mato Buntan’) was added to different media, and cell viability was monitored for several weeks using quantitative polymerase chain reaction (qPCR) in conjunction with ethidium monoazide (EMA). Among media tested, King’s B (K) did not support viability of LAS cells, while grapefruit juice (G) allowed LAS cells to survive *in vitro* for ~20 days. In media that sustained LAS viability, a reproducible biofilm-like substance was formed over time at the air-liquid interface of culture flasks and glass slides inserted in cultures. Fluorescence *in situ* hybridization (FISH) showed the biofilm contains aggregates of LAS cells, which was confirmed by qPCR. 16S rDNA libraries of the biofilm samples have been constructed and will be sequenced via Illumina next-generation sequencing to determine their bacterial composition. To elucidate why juice-based media prolongs LAS viability, the elemental nutrient compositions of the media and the biofilm were analyzed via inductively coupled plasma optical emission spectrometry (ICP-OES). Compositions were compared, and specific elements, such as potassium and calcium, were more abundant in media that sustain LAS cell viability. Results will contribute to future development of a culture medium for LAS.