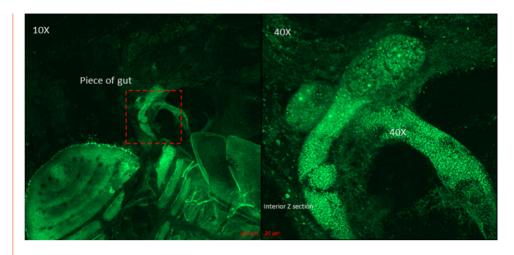
A Culturable L. crescens Model for Functional **Genomics of CLas**



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The primary reason for the lack of efficacious treatments to control HLB is likely due to lack of experimentally useful CLas cultures: at best only complex co-cultures can be obtained, containing such low CLas titers that they are detectable only by PCR. Potential CLas target genes necessary for psyllid acquisition, maintenance and transmission to citrus that might be useful to control CLas remain largely unknown and uninvestigated. All CLas strains lack key biosynthetic, metabolic and structural pathways that are found in Liberibacter

crescens (Lcr), which is axenically cultured. Our recently published data indicates that wild-type CLas will never be axenically cultured without adding all 95 Lcr genes that are missing in all CLas strains and required for growth (Cai et. al. 2022). To help identify CLas targets, a practical alternative to culturing is step-wise additions to Lcr of small blocks of CLas "symbiosis" genes needed for psyllid acquisition, replication and citrus transmission. Lcr is missing only 23 genes implicated as being required by CLas for psyllid infection. Additional genes

are published to be required to cause HLB in citrus. We have developed a functional genomics toolkit for practical manipulation of Lcr. including adding fluorescent markers (glowing Lcr are visualized inside psyllid gut 1 day after feeding by microscopy below) and CLas genes to Lcr. Our goal is to characterize CLas genes in Lcr that condition acquisition and transmission to citrus, but short of causing HLB. This will provide a platform for identifying critical gene targets for genetic and chemical controls.

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