8.3 Differential Expression of Potential Virulence Genes of Candidatus Liberibacter asiaticus in Infected Plants and Psyllids

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Candidatus Liberibacter asiaticus is the causal agent of citrus greening or huanglongbing, a destructive disease of citrus in the United States. Citrus greening is usually transmitted through grafting or through feeding by the Asian citrus psyllid (Diaphorina citri). In order to identify the potential virulence genes, real-time quantitative PCR assays using total RNA isolated from infected plants and psyllids were conducted to test the expression profile of Ca. L. asiaticus. Gene specific primers were used to check the expression of more than 500 genes in Ca. L. asiaticus. The genes showing a differential expression of two-fold or more in either the plant or psyllid were selected and categorized into COG (Clusters of Orthologous Groups of proteins) functional categories. Selected genes that were overexpressed in planta were further studied by expression or screened on Nicotiana benthamiana plants for symptom expression, using transient assays. Interestingly, one orf encoding salicylate hydroxylase (sahA) was identified in the genome of Las. Salicylate hydroxylase is responsible for salicylic acid (SA) breakdown. SA is important for basal defense, hypersensitive response, and systemic acquired resistance. Expression assays indicate SahA is functional and can break down various salicylate-based substrates. In addition, constructs AS7 and AS13, expressing two hypothetical proteins, caused symptoms on the plants and will be further characterized using transgenic expression studies on Duncan grapefruit (Citrus paradisi Macf.). Since the virulence mechanisms of Ca. L. asiaticus is poorly understood, the results from this study will serve as the first step in identifying potential virulence genes involved in symptom expression and survival of this pathogen in planta.