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'Candidatus Liberibacter asiaticus' Encodes Two Novel Autotransporters that Target to Mitochondria

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## 9.8

**‘*Candidatus Liberibacter asiaticus*’ Encodes Two Novel Autotransporters that Target to Mitochondria**Hao, G.<sup>1</sup>, Boyle, M.<sup>2</sup>, Zhou, L.<sup>1</sup>, and Duan, Y.<sup>1</sup><sup>1</sup>USDA-ARS-USHRL, Fort Pierce, Florida 34945<sup>2</sup>Smithsonian Marine Station, 701 Seaway Drive, Fort Pierce, FL 34949

As a phloem-limited, intracellular bacterial pathogen, ‘*Candidatus Liberibacter asiaticus*’ (Las) has a significantly reduced genome and causes huanglongbing (HLB), a devastating disease of citrus worldwide. In this study, we characterized two novel autotransporter proteins of Las, and redesignated them as LasA<sub>I</sub> and LasA<sub>II</sub> in lieu of the previous names Hyv<sub>I</sub> and Hyv<sub>II</sub>. Proteins secreted by the type V secretion system (T5SS), known as autotransporters, are large extracellular virulence proteins localized to the bacterial poles. Bioinformatic analyses revealed that LasA<sub>I</sub> and LasA<sub>II</sub> share the structural features of an autotransporter family containing large repeats of a passenger domain and a unique C-terminal translocator domain. When fused to the GFP gene and expressed in *E. coli*, the LasA<sub>I</sub> C-terminus and the full length LasA<sub>II</sub> were localized to the bacterial poles, similar to other members of autotransporter family. Despite the absence of the signal peptide, LasA<sub>I</sub> was found to localize at the cell surface by immuno-dot blot using a monoclonal antibody against the partial LasA<sub>I</sub> protein. Its surface localization was also confirmed by the removal of the LasA<sub>I</sub> antigen using a proteinase K treatment of the intact bacterial cells. When co-inoculated with a P19 gene silencing suppressor and transiently expressed in tobacco leaves, the GFP-LasA<sub>I</sub> translocator targeted to the mitochondria. This is the first report that Las encodes novel autotransporters that target to mitochondria. These findings may lead to a better understanding of the pathogenesis of this intracellular “energy parasitic” bacterium, and to more efficient characterizing new molecular targets for HLB control.