1.2 Genetic and Functional Characterization of the znu Operon in the Intracellular Citrus Pathogen, *Candidatus Liberibacter asiaticus*

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The uptake of zinc by *Candidatus Liberibacter asiaticus* (Las) may act as a virulence mechanism in its citrus hosts because symptoms of citrus huanglongbing often mimic those with zinc deficiencies. Annotation of the Las genome has revealed two putative operons, approximately 191 kb apart, each encoding for a high-affinity zinc uptake system. The operons contained either three or four genes consisting of znuACB or znuACBB, respectively. In this uptake system, ZnuA is predicted to function as a periplasmic metallochaperone, ZnuB as a membrane permease, and ZnuC as the ATPase subunit of the ATP-binding cassette transporter. Several motifs that are characteristic of these proteins, such as the Walker A and B motifs within ZnuC, have been identified in the Las encoded homologue. Interestingly, neither proteins homologous to those known to regulate this operon (zur) nor its regulatory elements (Zur box) have been identified in Las. To understand the role of these two operons in the fastidious Las bacterium, each individual gene was placed under an inducible promoter, transformed into the *Escherichia coli* BW25113 strain with the corresponding gene of interest knocked out, and analyzed in a complementation assay. Results from the assays have demonstrated that not all of the Las encoded genes appear to be able to functionally complement the *E. coli* knock-out strains. Because of the essential nature of zinc within the cell, this system may be crucial for the pathogen’s survival. Thus, disruption of this system may provide a possible mechanism for eliminating the bacterium.