Investigation of the Role of the Rna Binding Protein CsrA in the Emerging Pathogen *Escherichia albertii*

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**Abstract**

Attaching and effacing (A/E) pathogens are one of the main causes of severe infantile diarrhea and death in developing countries. A/E bacteria induce these effects by means of a type 3 secretion system (T3SS) that connects the bacterial cytosol to that of the host and enables effectors to be dispatched into the host cell where they alter its cytoskeleton, resulting in pedestal formation and the inability to properly absorb nutrients. Examples of A/E bacteria include enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and *Escherichia albertii* (*E. albertii*). In the majority of outbreaks involving *E. albertii*, the bacterium was initially misdiagnosed and later reclassified as the correct bacterium. This highlights the necessity of identifying as many unique traits of *E. albertii* as possible for correct classification.

The virulence of A/E pathogens is mainly controlled by a cluster of genes called the locus of enterocyte effacement (LEE). Although transcriptional regulation of these genes has been studied extensively, posttranscriptional regulation has not been largely examined. An example of posttranscriptional regulation includes the use of RNA-binding proteins to control mRNA stability and/or translation. One such protein, csrA, regulates mRNAs posttranscriptionally by directly binding to them. My research aimed at interrogating the role of the RNA binding protein *csrA* in *E. albertii*. I successfully inactivated *csrA* and found that it is involved in glycogen biosynthesis and regulation of the LEE. These results represent the first report to implicate CsrA in the virulence and metabolism in *E. albertii* - a bacterium that is severely understudied, although an increasing number of reports suggest this bacterium could be a cause of several outbreaks which were incorrectly attributed to other A/E pathogens. These results are critical for developing clinical measures to counteract this emerging pathogen.

**Summary**

- Western Blotting was used to examine the levels of the LEE-encoded Tir protein in the *csrA* mutant. The results revealed a decrease in the abundance of Tir in the mutant bacteria, suggesting that *csrA* is required for its synthesis.
- A glycogen biosynthesis assay demonstrated that glycogen production was increased in the *csrA* mutant in comparison to the wild type. This illustrated that *csrA* plays a role in suppressing glycogen synthesis.

**Future Studies**

- Analyze the role of *csrA* in biofilm dynamics utilizing a microtiter plate biofilm assay
- Use Western Blotting to probe for EspA, EspB, and EspD in the mutant bacteria
- Examine the quantity of the Tir, EspA, EspB, and EspD proteins excreted by the mutant bacteria

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**References**