Elucidating the Mechanism of Virulence in E. coli O104:H4: Identification of the Role of Biofilms in Stx-Phage Induction

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Escherichia coli O104:H4 is an enteroaggregative/shiga-toxin (Stx) producing strain and the cause of many fatal diseases. Recent literature identified a direct correlation between expression of genes associated with biofilm formation and virulence. However, the mechanism was unknown. Therefore, it was hypothesized that O104 biofilms induce lambda prophages, resulting in increased production of Stx and virulence. Transcriptional reporters were cloned into EcAB73 (a non-virulent lambda lysogen). Levels of cyclic di-GMP were elevated to induce production of biofilms. O104 mRNA and phage DNA isolates were used as templates for qRT-PCR and PCR. Target genes were q, stx and c1 and reference gene was rpoB. SOS promoters, RecN and SulA, were fused to GFP reporters and cloned into O104. The effects of PS on SF6 phage production and on various EAEC strain biofilm formation were examined. Results supported MMS and ciprofloxacin as being the most potent activators of the SOS response. stx gene copy number showed a higher phage concentration in biofilm populations. qRT-PCR showed an increase in q and stx expression and a decrease in c1 expression in biofilm populations. SF6 phage showed no changes in production dependent on PS concentration suggesting the viability of PS. Biofilm assays showed that EAEC biofilms were inhibited by PS as low as .00125%, expanding the role of PS as a biofilm inhibition agent. A higher level of RecN and SulA promoter activity was correlated to an increase in virulence gene expression. Statistical analysis was conducted on all data. Based on the results, O104 biofilms utilize lambda phages to increase their virulence. The identification of this mechanism allows for the development of novel therapeutics to target this pathway to cure O104 infections.

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