Fall 2012

Isolation and Phenotypic Characterization of Virulent UPEC LPS Mutagens

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Recommended Citation

http://openscholarship.wustl.edu/vol8_iss1/54

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Uropathogenic *Escherichia coli* (UPEC) predominates as the most common urinary pathogen, causing approximately 85% of urinary tract infections (UTIs). Its virulence is due mostly to its ability to form Intracellular Bacterial Communities within a host, rendering the body’s immune response ineffective, and can often lead to recurrent infections. This study involves the genetic analysis of two lipopolysaccharide (LPS) mutagens of UTI89 in an attempt to better understand the mechanisms involved in bacterial proliferation. Two LPS mutants, ΔWaaL and ΔWaaW, were obtained on kanamycin plates following electroporation of UTI89 PKM 208 competent cells with PCR products containing kanamycin-resistance cassettes for the regions of the WaaL and WaaW genes. Colonies for ΔWaaL and ΔWaaW had diameters of 4-5cm and 1-2cm, respectively. Biofilm assays performed on both mutants revealed similar biofilm formations to wild-type (UTI89) after 24 hours in LB, while significant biofilms were unable to form in YESCA media. This suggests that by affecting the LPS layer of UTI89, curli production may be suppressed. When colonies were streaked out onto Congo Red indicator plates, a color discrepancy between mutant and wild-type UTI89 was observed, further supporting a possible connection between LPS and curli formation. Immunoblots of ΔWaaL revealed that the WaaL gene in UTI89 encodes for an enzyme responsible for ligating the O-antigen to the lipid A-core of LPS due to the absence of banding on the developed immunoblot. Further testing is necessary to determine if a similar effect is exhibited in ΔWaaW. Curli Immunoblots with CsgA primary antibody, a major subunit of curli fibers, revealed that the mutants’ curli production remained unaffected when grown in LB. However, further testing is required to determine if curli remain present when strains are grown in YESCA media.