Unravelling the contribution of different surface factors in uropathogenic E. coli biofilm formation

M. A. Schembri; University of Queensland, Brisbane, AUSTRALIA.

Uropathogenic *Escherichia coli* (UPEC) are the primary cause of urinary tract infections, a significant disease increasingly associated with antibiotic resistance. UPEC pathogenesis and persistence in the urinary tract is a complicated process involving adherence, aggregation, extracellular and intracellular lifestyles, resistance to host innate immune factors and biofilm formation. The formation of biofilms by UPEC is linked to the coordinated expression of diverse surface factors including fimbriae (e.g. type 1 fimbriae and curli), autotransporter (AT) proteins (e.g. antigen 43 [Ag43]), polysaccharides (e.g. capsule) and flagella. These surface factors are subjected to various levels of regulatory control at different stages during biofilm development. We have studied the role of AT proteins in UPEC biofilm formation using a combination of molecular and structural approaches. Characterization of the functional passenger domain of the Ag43 AT protein revealed it adopts a twisted L-shaped beta-helical structure firmly stabilized by a three-dimensional hydrogen-bonded scaffold, and we have shown that this confirmation drives the formation of cell aggregates via a molecular Velcro-like handshake mechanism. Our more recent work has identified and mapped the interaction of a novel monoclonal antibody that inhibits Ag43-Ag43 interaction, yielding new insights into its function. In parallel work we have also shown that larger surface structures such as the polysaccharide capsule can mask Ag43 function. To understand the interplay between these surface structures, we devised novel strategy based on lytic capsule-specific phage killing, saturated Tn5 transposon mutagenesis and high throughput transposon directed insertion-site sequencing to define the entire complement of genes required for capsule production in UPEC. Our work identified new genes involved in the regulation of capsule expression, and suggest this regulatory control may intersect with the expression of other surface factors including AT proteins and flagella. Overall, this knowledge has informed the complex regulatory mechanisms that control the expression of key cell-surface factors that drive the development of heterogeneous UPEC biofilms.