

Examination of *fimB* transcriptional site point mutations on *fimB* transcription in uropathogenic *Escherichia coli*

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Uropathogenic *Escherichia coli* (UPEC) is the leading cause of human urinary tract infections. Type 1 pili encoded by *fim* genes are responsible for the attachment of UPEC to bladder cells of the urinary tract. We are interested in how point mutations in the *fimB* gene affect the ability of UPEC to transcribe *fimB* when grown in different environments (i.e. pH and osmotic stress) one might encounter in the human urinary tract. Previously, point mutations were made in two *fimB* transcriptional start sites. Bacteria were grown to mid-logarithmic phase in pH 5.5 or pH 7 buffered Luria broth (LB) with or without 400 mM NaCl. Total RNA was extracted from each strain, complementary DNA synthesized, and quantitative reverse transcribed-polymerase chain reaction analyses performed to quantitate the level of *fimB* transcription. A UPEC strain with a TATA box mutation in *fimB* second transcriptional start site displayed less *fimB* transcription compared to the unmutated *fimB* strain when grown under all conditions. Lower *fimB* transcription was observed in strains that contained first AC and higher affinity mutations in *fimB* second transcriptional start site versus the unmutated strain in cells grown in acidic pH/high osmolarity and neutral pH/low osmolarity LB media. Mutants that had third AC, higher affinity AC, or GAD box mutations displayed lower *fimB* transcription when cells were grown in neutral pH/high osmolarity LB media versus the unmutated strain. This study indicates the *fimB* second transcriptional start site is the main transcriptional start site that is engaged to drive *fimB* transcription in UPEC.