The Effects of pH and Osmolarity Conditions on the Type I Pili Expression Encoded by *fim* Genes

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) are known to be one of the leading causes of human urinary tract infections. Attachment to bladder epithelial cells leads to infection and is done through the use of pili. Several *fim* genes encode for type 1 pili, and previously it has been shown that environmental signals can affect their *fim* gene expression. This study was performed to confirm our previous results by constructing a *fimB-lux* fusion on a single copy plasmid, placing it into a UPEC strain, and testing *fimB* expression under different environmental conditions through luminescence measurements. The results showed that *fimB* expression was repressed when the *E. coli* were grown in acidic media compared to neutral pH. Furthermore, grown in high osmolarity media actually increased *fimB* expression significantly, resulting in conflicting data compared to past *in vitro* results. From the data, both acidic and osmolarity conditions appear to affect the expression of *fimB* and in turn type 1 pili expression. Eventually, this *fimB-lux* system will be tested in *E. coli* growing in the urinary tracts of mice.

INTRODUCTION

Urinary tract infections (UTIs) afflict thousands of women each year and uropathogenic *Escherichia coli* (UPEC) are the primary cause of these infections (Valiquette, 2001). These infections lead to high health costs that are over a billion dollars per year. Pili present on the bacterial cells help to initiate these infections and more specifically, type 1 pili, are primarily responsible for the attachment of *E. coli* to bladder epithelial cells in humans (Schilling et al, 2001). Bacterial cells can switch between whether or not the pili are expressed by a process called phase variation (Schwan et al, 2002). It is believed that the environment in which the bacteria reside plays a role in the phase variation process.

There are two *fim* genes; *fimB* and *fimE,* whose protein products regulate expression of the structural gene, *fimA*, needed for type 1 pilus expression. Previous work examined how pH, osmolarity, and human urine affected transcription of the *fimB* and *fimE* genes (Schwan et al, 2002). However, this study was of limited use because they were performed in test tubes, not in animals. In an attempt to answer whether *fimB* expression was repressed in bacteria growing in the urinary tract, a *fimB-lux* fusion was created and transferred to a single copy number plasmid. Initial *in vitro* characterization was then performed.

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions

E. coli strain DH5α was used for transformations. Plasmid pP5-8 contains the *fimB* promoter flanking *EcoRI* and *BamHI* restriction sites and ampicillin resistant site. The pPP2-6 single copy number plasmid has a chloramphenicol resistant site and the pHSS22 plasmid contains a kanamycin resistant site.

Construction of the fimB-lux fusion

Plasmid pP5-8 containing the *fimB* transcriptional start site was cut with *EcoRI* and *BamHI* restriction endonuclease enzymes. This *fimB* DNA was then fused to pHSS22 plasmid DNA and also digested with *EcoRI* and *BamHI*. After this fusion, the fused DNA was used to transform *E. coli* strain DH5α cells, selecting for growth on Luria agar (LA) containing kanamycin. This created the pWS141-2 plasmid. Next, a promoterless (no transcriptional start site) *lux* gene was cut out of a plasmid called pXen5 using *BamHI.* This *lux* gene encodes for bioluminescence (glow in the dark) and was ligated to pWS141-2 plasmid DNA that had been cut with *BamHI*. The ligation mix was used to transform *E. coli* strain DH5α cells and colonies on LA with kanamycin were screened for bioluminescence. One plasmid clone was identified, named pWS144-27, was cut with *NotI* and the *fimB-lux*

containing piece of DNA was ligated to *NotI* digested pPP2-6. The ligated DNA was used to transform DH5α cells, selecting for colonies on LA that were bioluminescent and resistant to chloramphenicol. One clone resulted, named pWS145-5, which was used for *in vitro* characterization.

In vitro bioluminescence assays

The *E. coli* strain with the pWS145-5 plasmid, containing a *fimB-lux* fusion, was grown in LB media with variations of pH and osmolarity. Luminescence assays were then performed to measure the effects of the growth environment on the *fimB* gene when the bacteria were propagated in a test tube. Strains DH5α/pWS145-5 and DH5α/pWS145-38 were grown in LB with a pH of 5.5 with or without 400 mM NaCl (high salt) compared to a neutral pH with or without 400 mM NaCl. The results were tabulated by dividing the RLU relative luminescence units by the OD_{600} reading of the culture.

RESULTS

Examination of the *fimB-lux* fusion at different pHs and osmolarities. In order to determine whether the pH affected the transcription of the *fimB* gene, a fusion of the *fimB* promoter with the *lux* gene was generated. The *fimB-lux* fusion was effectively cloned into a single-copy number plasmid, resulting in pWS145-5 and pWS145-38 and then transformed into *E. coli* strain DH5α.

Luminescence measurements were taken when the cells were in mid-log phase, which was determined by optical density. Higher luminescence activity was observed when cells were grown in media of pH 7.0 for both single-copy number plasmids compared to pH 5.5 (FIG. 1). When both strains were grown in media of pH 7.0 with the addition of 400 mM NaCl, expression of the *fimB* gene was higher with 400 mM NaCl compared to the assays noted above. Furthermore, an examination of growth media at pH 5.5 with 400 mM NaCl also showed higher levels of expression as compared to the no salt tester.

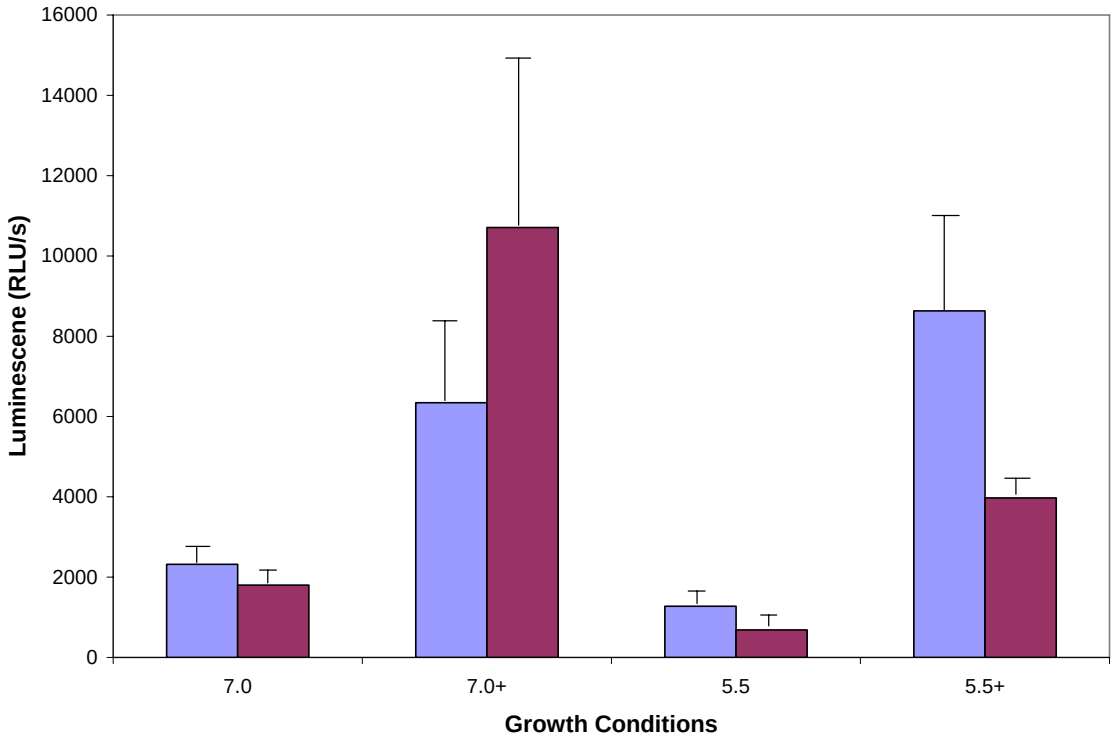

FIG. 1. Luminescence assays of strains DH5α/pWS145-5 (left bar) and DH5α/pWS145-38 (right bar) cells grown in LB at pH 5.5 or 7.0 ± 400 mM NaCl with means and ± standard deviations shown. Each data point represents at least three separate runs.

DISCUSSION

The effects of pH and osmolarity on the expression of the *fimB* gene were studied through the use of the *lux* gene by measuring luminescence. Transcription of the *fimB* gene tested was repressed under acidic conditions but actually increased under osmolarity conditions with NaCl acting as the osmolyte. Previous work done by Schwan et al. (2) had shown that both acidic conditions as well as osmolarity conditions repressed the *fimB* gene when using β-galactosidase to measure the activity. The *lux* gene may be affected differently under osmolarity conditions resulting in the conflicting results.

The *lux* gene may need to be studied on its own, in order to determine if it is affected differently than the *fimB* gene normally would by itself, to obtain accurate results when used *in vivo* studies. With conflicting results from the study done with β-galactosidase, the actual effect of osmolarity conditions on the *fimB* gene would have to be determined prior to *in vivo* studies for correct results.

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