

## A Mutagenesis Study to Find *fimE* Regulating Genes in *Escherichia coli*

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### ABSTRACT

The bacterial species *Escherichia coli* is responsible for the majority of urinary tract infections in humans, in part due to attachment of bacteria to uroepithelial cells via type 1 fimbriae. This study focused on one gene involved in type 1 pilus expression, *fimE*. FimE regulates expression of type 1 fimbriae by changing the position of the structural gene's promoter to prevent transcription. To determine what genes may be involved in regulating *fimE*, an *E. coli* strain lacking *fim* and *lac* genes was transformed with a plasmid containing the *fimE* promoter hooked to a promoterless *lacZ* gene. The aims of this study were: 1) to randomly insert transposons into genes within the genome of this strain, 2) to screen the mutants generated for their ability to affect *fimE* expression as measured by the amount of  $\beta$ -galactosidase produced, and 3) to identify by DNA sequencing one or more genes affecting *fimE* expression. A total of 554 random transposon mutants were generated and screened for their ability to affect *fimE* expression. Of these mutants, three reproducibly showed down-regulation of *fimE*, and one showed up-regulation. Further testing will be needed to characterize the nature of their behavior, and eventual DNA sequencing will elucidate which exact genes were mutated.

### INTRODUCTION

In order for uropathogenic *Escherichia coli* (UPEC) to sustain an infection in the human bladder, the bacteria can attach to mannose containing receptors on uroepithelial cells via binding by their type 1 fimbriae. Expression of these type 1 pili is mediated by several *fim* genes and involves a process called phase variation (1), where cells can switch from being piliated to non-piliated and vice versa. One of the genes involved in this switching, *fimE*, exclusively turns off expression of type 1 pili by flipping the orientation of a 314-base pair invertible DNA element that contains the structural gene promoter for type 1 pili into an off orientation (2, 3, 4). A previous study showed that *fimE* transcription is activated when bacterial cells are grown in a low pH high osmolarity environment commonly found in the kidneys of a human (5).

In this study, random mutations were induced in a strain of *E. coli* via a transposon mutagenesis, generating 554 mutant colonies. These mutants were screened for their effect on *fimE* expression. Three of these mutants have shown deactivation of the gene, and one has shown derepression. Further characterization of these mutants will ascertain whether their defects are pH or osmolarity related. DNA sequencing could possibly indicate previously unknown genes that may be involved in overall regulation of *fimE* expression at the molecu-

lar level. The significance of this work will be critical in deciphering how type 1 pilus expression is regulated, which could play a role in preventing or eliminating urinary tract infections.

## MATERIALS AND METHODS

**Bacterial strains, media, growth conditions.** The bacterial strains used in this study included *E. coli* strain AAEC189, which exhibits resistance to kanamycin and has all *fim* and *lac* genes deleted within the genome, and *E. coli* strain S17.1 $\lambda$ *pir*, that has the *λpir* function to allow replication of plasmids with the R6K origin and resistance to streptomycin. Both *E. coli* strains were grown in Luria-Bertani (LB) broth at 37°C or passaged on LB agar plates incubated at 37°C. The following antibiotics were used at the following concentrations: tetracycline (Tc), 12.5 μg ml<sup>-1</sup>, kanamycin (Km), 40 μg ml<sup>-1</sup>, and chloramphenicol (Cm), 10 μg ml<sup>-1</sup>.

**Plasmids.** The plasmid pJLE4-3 contains a chloramphenicol resistance gene and has the *fimE* promoter hooked up to a promoterless *lacZ* reporter gene. Plasmid pUT-Tc, contains a mini-Tn5 transposon with a tetracycline resistance gene and an *oriT* site that will allow a conjugal bridge to form between bacterial cells.

**Conjugal Mating and Transposon Mutagenesis.** *E. coli* strain AAEC189, transformed with pJLE4-3, and S17.1 $\lambda$ *pir*, transformed with pUT-Tc, were grown to mid-log phase at 37°C in LB. Aliquots of each population were mixed in a 1.5 ml microfuge tube and then spotted onto a LA plate containing no antibiotics. For each mating, the plate was incubated at 37°C overnight, the circle of growth on the plate was harvested into 3 ml of LB, and aliquots of the conjugated cells were spread onto LA plates containing TcKmCm. AAEC189/pJLE4-3 cells that had successfully had the mini-Tn5 (Tc) transposon transferred into their genomes displayed resistance to chloramphenicol, tetracycline, and kanamycin.

**β-galactosidase Assays.** Colonies arising on the LA + TcKmCm plates were inoculated into the wells of 2-ml deep 96-well microtiter plates. These mutants were grown overnight in pH 5.5 LB containing 400 mM NaCl, then reinoculated into another set of plates using 50 μl aliquots of the original cultures. The mutants were allowed to grow to mid-logarithmic phase, the cells were permeabilized with sodium dodecyl sulfate and CHCl<sub>3</sub>, and β-galactosidase assays were performed by the method of Miller (5). As a control, strain AAEC189/pJLE4-3 cells that had not undergone transposon mutagenesis was used. Mutants showing greater β-galactosidase expression (derepression) or less β-galactosidase expression (deactivation) compared to the control strain were examined further. Sets of test tubes containing 5 ml of LB set at pH 5.5 with 400 mM NaCl were inoculated with each individual mutant and grown to mid-log phase. Once this level of growth was reached, β-galactosidase assays were done as described above. Assays were performed three different times on different days. The data was expressed as the mean ± standard deviation of these measurements. Those mutants showing continual deactivation or derepression of *fimE* were further examined for their effects on β-galactosidase expression in test tubes containing 5 ml of LB, pH 7.0. Assays were again performed three different times on different days and data was expressed as the mean ± standard deviation of these measurements.

## RESULTS

As seen in Table 1, initial  $\beta$ -galactosidase screens on the 554 transconjugants generated several mutants with either an increase or decrease in *fimE* expression compared to control strain *Escherichia coli* AAEC189/pJLE4-3.

**Table 1. Screening of  $\beta$ -galactosidase expression in Tn5 transposon mutants grown in pH 5.5 LB containing 400 mM NaCl compared to unmutated AAEC189/pJLE4-3 cells**

Phenotype <sup>a</sup>	Number of Mutants
No difference	509
Higher $\beta$ -gal	15
Lower $\beta$ -gal	30

<sup>a</sup> Phenotypes were categorized as no difference (within 25  $\beta$ -gal Units from the control), higher  $\beta$ -galactosidase (when greater than 25  $\beta$ -gal Units of the control), or lower  $\beta$ -galactosidase (when less than 25  $\beta$ -gal Units of the control).

As seen in Table 2, further  $\beta$ -galactosidase screening identified three mutants that were no longer *fimE* activated and one that was no longer *fimE* repressed.

**Table 2.  $\beta$ -galactosidase activity values for individual mutants with deactivating and derepressing phenotypes grown in differing pH and osmolarity environments, compared to unmutated AAEC189/pJLE4-3 cells**

Mutant	# Miller Units at pH 5.5 LB and 400mM NaCl <sup>a</sup>	Miller Units at pH 7.0 <sup>a</sup>
Control <sup>b</sup>	124 $\pm$ 11	232 $\pm$ 22
4	74 $\pm$ 20	158 $\pm$ 16
103	76 $\pm$ 31	192 $\pm$ 22
320	82 $\pm$ 7	203 $\pm$ 21
392	138 $\pm$ 39	306 $\pm$ 28

<sup>a</sup> Miller Units were calculated according to Miller (5), representing the mean  $\pm$  standard deviation from three assays.

<sup>b</sup> Control strain was AAEC189/pJLE4-3.

## DISCUSSION

Previous studies have shown that an environment with low pH combined with moderate to high osmolarity favors transcription of *fimE* and thus inversion of the structural gene's promoter, resulting in non-piliated cells (6). In this study, *fimE* expression in Tn5 mutated *E. coli* strains was compared to the unmutated *E. coli* strain AAEC189/pJLE4-3. In LB pH 5.5, 400 mM NaCl medium, three mutants were identified that showed deactivation of the gene, and one that showed derepression. These findings suggest that in the three mutants showing *fimE* deactivation, a gene(s) has been mutated whose product is normally associated with *fimE* activation under these environmental conditions, and in the mutant showing *fimE* derepression, a gene has been mutated whose product is normally associated with *fimE* repression. By studying all of these mutants for their effect on *fimE* expression in pH 7.0 LB with no added NaCl, they displayed similar phenotypes as before, with the exception of mutant 392 which

showed a significant increase in its degree of *fimE* depression. Further study involving their phenotypic effects and overall growth at pH 7.0 and 5.5 over a range of osmotic conditions, compared to unmutated AAEC189/pJLE4-3, will better characterize the nature of their mutations and give insight as to whether or not they affect the organism's overall ability to survive. Eventual DNA sequencing will determine which genes have been mutated, possibly leading to the discovery and elucidation of a new gene(s) involved with *fimE* expression in UPEC.

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## REFERENCES

1. **Eisenstein, B.I.** 1981. Phase variation of type 1 fimbriae in *Escherichia coli* is under transcriptional control. *Science* **214**:347-349.
2. **Klemm, P.** 1986. Two regulatory *fim* genes, *fimB* and *fimE*, control phase variation of type 1 fimbriae in *Escherichia coli*. *EMBO J.* **5**:1389-1393.
3. **McClain, N.S., I.C. Blomfield, K.J. Eberhardt, and B.I. Eisenstein.** 1993. Inversion-dependent variation of type 1 fimbriae in *Escherichia coli*. *J. Bacteriol.* **175**:4335-4344.
4. **McClain, N.S., I.C. Blomfield, and B.I. Eisenstein.** 1991. Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. *J. Bacteriol.* **173**:5308-5314.
5. **Miller, J.H.** 1972. *Experiments in Molecular Genetics*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
6. **Schwan, W.R., J.L. Lee, F.A. Lenard, B.T. Matthews, and M.T. Beck.** 2002. Osmolarity and pH growth conditions regulate *fim* gene transcription and type 1 pilus expression in uropathogenic *Escherichia coli*. *Infect. Immun.* **70**:1391-1402.