

# Examination Of Environmental Conditions that Affect *fimA* Gene Expression in *Escherichia coli*

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## ABSTRACT

Uropathogenic *Escherichia coli* are a significant cause of urinary tract infections because of their ability to bind to uroepithelial cells. Long, thin appendages called type 1 pili mediate this adherence. The structural gene coding for the pilin monomer units is *fimA*. This *fimA* gene has an invertible element associated with the expression of the gene. When the invertible element is in the "ON" position, transcription of *fimA* occurs, whereas being in the "OFF" position results in a lack of transcription and a nonpilated phenotype. Two plasmids containing a *fimA-lacZ* fusion were tested to try to ascertain how the *fimA* gene is regulated when *E. coli* cells are grown under different osmotic conditions. The first plasmid was a multicopy number plasmid containing the *fimA* promoter locked in the "ON" position. The second plasmid was a single copy number plasmid containing the *fimA* promoter locked in the "ON" position. High osmolarity did not affect transcription of the *fimA* gene in the multicopy number plasmid, while high osmolarity increased transcription of the *fimA* gene in the single copy number plasmid.

## INTRODUCTION

The bacterial species *Escherichia coli* is responsible for 80-90% of all urinary tract infections(1, 2). Bacterial adherence to urinary tract epithelial cells is a critical step in the onset of a urinary tract infection. The bacterial cell structures primarily responsible for this adherence are tiny thread-like appendages called pili. *E. coli* produce a number of different types of pili, but type 1 pili have been shown to bind to bladder epithelial cells lining the urinary tract (4). Because type 1 pili have been shown to be responsible for adherence of *E. coli* cells to bladder epithelial cells, it is important to understand what regulates their expression.

Several *fim* genes are involved in the production of type 1 pili (4). Expression of type 1 pili involves a process known as phase variation where individual *E. coli* cells can switch from a cell expressing type 1 pili (being in the "ON" position) to a cell that no longer expresses these structures (being in the "OFF" position). This phase variation is the result of the positioning of an invertible element located upstream of the *fimA* structural gene. This *fimA* gene, encodes for the building blocks of the type 1 pilus structure. The transcriptional start site for *fimA* is located on the invertible element. When the *fimA* promoter is in the "ON" position it will allow transcription of the *fimA* gene, resulting in expression of type 1 pili.

In this study, two plasmids containing the *fimA* promoter locked in the "ON" position were transferred into *E. coli* cells lacking any *fimA* genes. One plasmid was a multicopy number plasmid, whereas the other was a single copy number plasmid. These recombinant cells were then grown under different osmotic conditions to see how *fimA* transcription was affected.

## MATERIALS AND METHODS

### Bacterial strains, plasmids, and growth conditions

Two strains were used in this study, *E. coli* AAEC189 ( $\Delta$ fm  $\Delta$ lac) provided by Ian Blomfield, Wake Field University and *E. coli* DH5a (*lacA* mutation) provided by Gibco/BRL, Gaithersburg, MD. Plasmids pAON-1 and pPP2-6 were supplied by James Duncan, Northwestern University. The plasmid pWS124-17 was made using the plasmids pAON-1 and pPP2-6 as described below. Both of the strains were grown in Luria-Bertani (LB) broth at 37 C or passaged on LB agar (LA) plates incubated at 37 C. The following antibiotics (from Sigma Chemical Co., St. Louis, MO) were used at the following concentrations: ampicillin, 100 mg ml<sup>-1</sup>, and chloramphenicol, 12.5 mg ml<sup>-1</sup>.

### Transformation of pAON-1 into AAEC189

The plasmid pAON-1 was isolated using a commercial kit (Qaigen) and the product size (6.1 kb) verified on a 0.8% agarose gel using a commercial molecular weight marker (Gibco/BRL) for comparison. The plasmid DNA was then transformed into *E. coli* strain AAEC189 and plated onto LA containing ampicillin according to Sambrook et. al. (5). Several transformants were isolated, plasmid extractions performed, and the plasmid verified in these clones. One of these recombinant AAEC189/pAON-1 clones was used through the rest of the study.

### Creation and transformation of pWS124-17

Plasmid DNA from pAON-1 and pPP2-6 were digested with the restriction endonuclease NotI overnight at 37 C. Both plasmid DNA's were then ligated and transformed into competent *E. coli* DH5 $\alpha$  cells. The transformed cells were plated onto LA that contained chloramphenicol and X-Gal. A functional *lacZ* gene will produce  $\beta$ -galactosidase which will cleave X-Gal and impart a blue color to the colonies. A blue colony was picked, the plasmid DNA was isolated, and a NotI digestion was performed for confirmation. The plasmid was named pWS124-17. The plasmid was then transformed into the strain AAEC189 (5).

### The $\beta$ -galactosidase assay.

Overnight cultures of AAEC189/pPP2-6, AAEC189/pAON-1, and AAEC189/pWS124-17 in LB broth were grown to mid-logarithmic phase at 37 C with shaking in pH 7.0 LB broth with or without 400 mM NaCl. Three hundred microliter aliquots of bacteria were permeabilized with sodium dodecyl sulfate and CHCl<sub>3</sub>, then assayed for  $\beta$ -galactosidase activity using the method of Miller (3). Measurements were performed after a 20 minute exposure to the substrate. The data was expressed in Miller Units of  $\beta$ -galactosidase activity.

**Table 1.** Beta-galactosidase activity in the *E. coli* strain AAEC189 transformed with different recombinant plasmids and grown under different osmotic conditions.

Plasmid	Condition	$\beta$ -galactosidase <sup>b</sup>
pPP2-6	LB pH 7.0	0
pPP2-6	LB pH 7.0 + <sup>a</sup>	0
pAON-1	LB pH 7.0	545 $\pm$ 176
pAON-1	LB pH 7.0 +	408 $\pm$ 8
pWS124-17	LB pH 7.0	654 $\pm$ 10
pWS124-17	LB pH 7.0 +	1014 $\pm$ 23

<sup>a</sup> + = LB + 400 mM NaCl

<sup>b</sup>  $\beta$ -galactosidase activity calculated in Miller units. These numbers are the mean and standard deviation from two separate assays.

## RESULTS AND DISCUSSION

### Testing the multicopy pAON-1 plasmid for $\beta$ -galactosidase expression

A *fimA-lacZ* reporter fusion had been previously created on the multicopy plasmid pAON-1 that was provided by James Duncan. The expression of *fimA* could be influenced by both the positioning of the invertible element as well as some factor affecting the *fimA* promoter itself. To determine if the environment was affecting the latter, the invertible element was locked in the “ON” position, thus the invertible element should not be able to switch to the “OFF” position in this genetic construct. This recombinant plasmid was moved into the AAEC189 strain background because this strain is  $\Delta fim$  and  $\Delta lac$ , so there should be no interference in the  $\beta$ -galactosidase assays. Two separate  $\beta$ -galactosidase assays were performed with the AAEC189/pAON-1 bacteria, growing the bacteria in LB with or without 400 mM NaCl. The results showed that there was no significant difference in the amount of transcription from the *fimA* promoter in the low osmotic growth conditions (545 Miller Units) compared to the high osmotic (400 mM) growth conditions (408 Miller Units; Table 1). The standard deviation was very high for the cultures grown without added salt, which could explain the lack of a significant difference.

The amount of transcription of the *fimA* gene found in this study is significantly higher than the amount of transcription found in a study done by Schwan et. al. in preparation this was expected because the plasmid in this study was a multicopy plasmid that contained a promoter locked in the “ON” position for *fimA*, while Schwan’s study used a low copy number plasmid that did not contain a locked promoter. In this study the invertible element was able to switch positioning, causing the promoter to be turned “ON” or “OFF”, resulting in less transcription of the *fimA* gene.

### Creating and testing a low copy number plasmid containing the *fimA* promoter locked in the “ON” position

Because the results with the multicopy plasmid construct could be misleading due to multicopy effects, the *fimA* promoter locked in the “ON” position was cloned into the single copy number plasmid pPP2-6. This recombinant plasmid was also transferred into the AAEC189 background to get a better assessment of the regulatory events affecting *fimA*

expression. Two separate  $\beta$ -galactosidase assays were done with the AAEC189/pWS124-17 strain and compared with the AAEC189/pPP2-6 strain that served as a control in these assays. When AAEC189 was grown in LB without added NaCl, the level of  $\beta$ -galactosidase expression was higher than for the multicopy plasmid pAON-1 (Table 1). Moreover, the level of  $\beta$ -galactosidase expression was more than double for the single copy number plasmid pWS124-17 when the AAEC189 cells were grown in LB containing 400 mM NaCl. With a significance of  $p < 0.003$ . The control strain AAEC189/pPP2-6 had no  $\beta$ -galactosidase expression as expected.

A possible explanation for transcription of the *fimA* gene in the clone AAEC189/pWS124-17 (low copy number) being much higher than in the clone AAEC189/pAON-1 (high copy number) is maybe due an activator protein. Although there are more copies of the plasmid in AAEC189/pAON-1 than in AAEC189/pWS124-17 there might only be one activator protein to activate transcription. One activator protein could explain why there is not more transcription in AAEC189/pAON-1 than in AAEC189/pWS124-17.

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