

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE REGULATION OF *fimB* INVOLVED IN TYPE 1 PILUS EXPRESSION

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ABSTRACT

The bacterial species *Escherichia coli* causes most urinary tract infections. Adherence to bladder cells via thin appendages called pili are important in causing these infections. One variety of pili is type 1 pili that can undergo phase variation, allowing the bacteria to switch between piliated and nonpiliated states. Phase variation involves several genes, which include *fimA*, that encodes for the major subunits of type 1 pili and two other genes named *fimB* and *fimE*. These last two genes encode for the two DNA binding proteins, FimB and FimE, which contribute to expression of *fimA* and subsequent formation of the pili. The environment may play a role in the expression of these *fim* genes. To ascertain how the environment may regulate type 1 pilus expression, a *fimB::lacZ* fusion was generated on a single copy number plasmid. This fusion was created by connecting part of the *fimB* gene involved in initiating transcription (i.e. the promoter) to a promoterless β -galactosidase gene, *lacZ*. The amount of β -galactosidase activity will be affected by the activation of the *fimB* promoter that will in turn lead to more β -galactosidase being expressed and more conversion of the added substrate. As the *fimB* gene is shut down, less *lacZ* is expressed so less substrate is broken down and reduced color occurs. The *E. coli* cells with plasmid-borne *fimB::lacZ* were assayed under different pH and salt conditions. As salt concentration increased, *fimB* expression decreased, whereas when pH decreased, *fimB* expression also decreased. These results suggest that pH and salt found in human urine can affect *fimB* expression and possibly type 1 pilus expression.

INTRODUCTION

The bacterial species *Escherichia coli* is responsible for numerous diseases in humans. An estimated 7 million American women see their doctors each year for bladder infections and 80-90% of these infections are caused by *E. coli* (2, 3). Adherence to bladder epithelial cells is a critical step in the onset of a bladder infection (6). This adherence can be mediated by pili, which are tiny thread-like appendages found extending outward from the outer bacterial surface. *E. coli* has many pilus types, but type 1 pili have been shown to promote bacterial persistence. In addition, type 1 piliated *E. coli* causes symptoms to appear quicker and last longer (1). Type 1 piliated bacteria can bind to a variety of eukaryotic cells, includ-

ing those lining the urinary tract and oropharyngeal region of a human host (5). A correlation exists between the expression of this pilus type by the bacteria and the potential to cause cystitis and urethritis (5). Because the type 1 pili appear to be important in uropathogenic *E. coli*, the study of what regulates their expression is important.

A unique feature of type 1 pili is that their expression can be regulated at the genetic level by a phenomenon known as phase variation. This refers to the bacteria's ability to switch between the piliated phenotype and a naked nonpiliated state (5). What is affected at the genetic level during this phase variation is the expression of the *fimA* gene, encoding for the major structural subunit of type 1 pili. The *fimA* promoter, or area that controls transcription of *fimA*, is located on an invertible DNA element. When the *fimA* promoter is aligned to allow transcription of *fimA*, or is in the "on" orientation, the cells are piliated. Conversely, if this element is switched to the opposite, or "off" orientation, transcription of *fimA* does not occur and the cells are nonpiliated. Positioning of this invertible element is affected by two DNA binding proteins, FimB encoded by the *fimB* gene and FimE encoded by the *fimE* gene. Both of these proteins bind to the invertible element. FimB binding allows positioning of the element to the "on" or "off" position, whereas FimE binding places the invertible element in the "off" position and prevents expression of the *fimA* gene (5).

In this study, a *fimB::lacZ* fusion was constructed and transferred into *E. coli* cells. These recombinant *E. coli* were grown under different environmental conditions to see how they affected *fimB* expression, which in turn would affect the production of type 1 pili.

MATERIALS AND METHODS

Bacterial strains, plasmids and growth conditions

E. coli strains DH5 α and HB101 were the background strains for transformations with the *fimB* recombinant plasmid. The plasmid pUTE4; provided by James Duncan, Northwestern University; contains a *fimB::lacZ* fusion that is flanked by *NotI* sites. Plasmid pPP2-6, also provided by James Duncan, is a single copy number plasmid derived from pPR274. This plasmid possesses a chloramphenicol resistance gene, an *oriT* site, and an origin of replication that is limited to single copies per cell. All *E. coli* cells were grown on Luria-Bertoni (LB) media (Giblo/BRL, Gaithersburg, MD) supplemented with chloramphenicol (Sigma Chemical Co., St. Louis, MO) at a concentration of 12.5 $\mu\text{g}/\text{ml}$. For the combined pH and osmolarity studies, the bacteria were grown in LB + 1% glycerol (vol/vol) buffered to the proper pH with 0.1 M NaPO₄ buffer. To achieve the respective osmolarities, the base buffered LB was added to buffered LB at 1 M NaCl to achieve osmolarities of 100 mM, 200 mM, 400 mM, and 800 mM.

Construction of the *fimB::lacZ* recombinant plasmid

Plasmid pPUTE4 was digested with *NotI* and the DNA fragment was ligated to a *NotI* digested single copy number plasmid called pPP2-6. Following the ligation of the two pieces of DNA, the closed plasmid that formed was transferred into *E. coli* DH5 α cells via transformation of the naked DNA, and the transformed cells were plated onto Luria agar that contained 12.5 $\mu\text{g}/\text{ml}$ of chloramphenicol and X-Gal. A functional *lacZ* gene will express β -galactosidase that will cleave X-Gal into galactose and X, which will impart a blue color to the colonies. Several blue colonies were picked, plasmid DNA isolated, and *NotI* restriction digests performed on the extracted DNA. From this screening, the plasmid named pJB5A was chosen. This plasmid was now transformed into *E. coli* strain HB101 cells and β -galactosidase assays were performed on cells grown under different conditions.

The β -galactosidase assay.

Overnight cultures of HB101/pJB5A and HB101/pPP2-6 (control strain) were grown in Luria broth (LB) and then passaged into LB with varying salt concentrations or pH readings. The cells were grown on a shaker until the OD_{600} was between 0.5 and 1.0. Two hundred microliter aliquots of cells were added to Z buffer (0.06 M $Na_2HPO_4 \cdot 7H_2O$, 0.04 M NaH_2PO_4 , 0.01 M KCl, 0.001 M $MgSO_4 \cdot 7H_2O$, and 0.05 M β -mercaptoethanol). Chloroform and 0.1% sodium dodecyl sulfate were added to each tube and vortexed to solubilize the cell membranes. The cells were then allowed to incubate in a 28°C water bath for five minutes. The enzymatic reaction was started by adding 200 μ l of a 4 mg/ml solution of the substrate ortho-nitro-phenyl-galactose in 0.1 M sodium phosphate buffer, pH 7.0, and the mixture was incubated for 25 minutes at room temperature. The reaction was stopped with 500 μ l of 1 M sodium bicarbonate. Aliquots were placed in a spectrophotometer to read at an OD_{550} and OD_{420} . From these readings, the units of β -galactosidase activity were calculated according to Miller (4).

RESULTS AND DISCUSSION

Three separate β -galactosidase assays were performed on HB101/pJB5A cells on separate days. The HB101/pPP2-6 strain served as a negative control. The cells were grown in LB supplemented with NaCl at concentrations of 100 mM, 200 mM, 400 mM, and 800 mM. Luria broth without added salt served as a control. All of the culture media was adjusted to a pH of 7.0. The expression of *fimB* was the highest at 100 mM of salt and decreased with higher concentrations of salt. The pPP2-6 exhibited only background levels of β -galactosidase activity (Figure 1). The same ranges of salt concentrations were next tested on a *fimB::lacZ* recombinant strain at a pH of 5.5. The HB101/pPP2-6 strain again served as a negative control and showed only background levels of β -galactosidase activity. As the level of NaCl increased, *fimB* expression fell precipitously, more so than when the pH was at 7.0 (Figure 2).

These results give us a better understanding of the role of type 1 pilus expression in causing urinary tract infections. The fact that different pH and salt concentrations affect the expression of *fimB* can allow for better control of urinary tract infections by changing the pH or salt concentration in the body. This could possibly be accomplished by changing one's diet. Since *fimB* expression causes the pili to turn "on", it could also be a target for new medications. A drug that could shutdown *fimB* expression would subsequently prevent pilus formation, thus controlling the urinary tract infection without the side effects that may occur by changing a person's diet.

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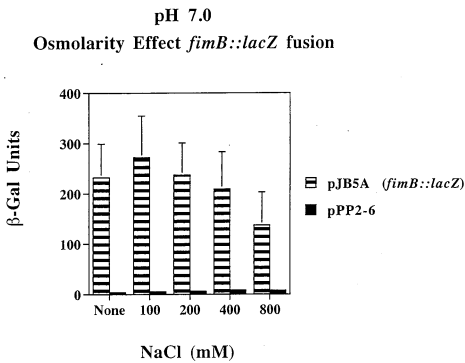


FIG 1. The data represented is the mean \pm the standard deviation of three separate β -galactosidase assays of *E. coli* strain HB101 cells containing pJB5A (*fimB::lacZ*) grown in Luria broth adjusted to a pH of 7.0 in different concentrations of NaCl. *E. coli* strain HB101 cells containing pPP2-6 were used as a negative control.

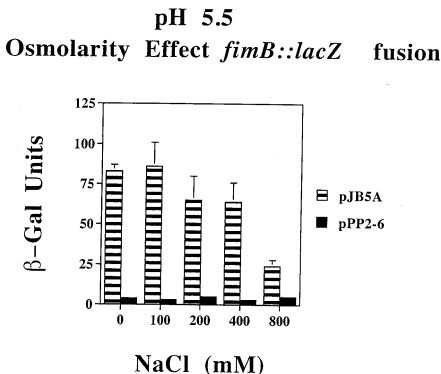


FIG 2. The data represented is from three β -galactosidase assays of *E. coli* cells containing the *fimB::lacZ* fusion. The cells were grown in Luria broth adjusted to a pH of 5.5 containing different concentrations of NaCl. The *E. coli* cells containing the pPP2-6 plasmid were used as a negative control.