USING FLUORESCENT UPEC AS A MODEL TO STUDY INTERACTIONS WITH HUMAN BLADDER CELLS

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Abstract

Escherichia coli (*E. coli*) are opportunistic bacteria that reside in the intestines of humans and contribute to gastrointestinal health. However, there are some strains that can cause a variety of diseases including urinary tract infections (UTIs). UTIs caused by *E. coli* are the most common type of bacterial infections seen in women and are a significant public health concern. Uropathogenic *E. coli* (UPEC) have acquired specific virulence factors including adhesins and fimbriae, which lead to increased adherence and invasion into urinary tract cells in the host. The pathogenic mechanisms employed by UPEC that promote adhesion and invasion have yet to be fully elucidated. We propose to study the mechanisms of adherence and invasion of UPEC to host cells by generating UPEC expressing the green fluorescent protein (GFP). we hypothesize that the GFP-expressing UPEC will assist in studying host-pathogen interactions. To test this hypothesis, we transformed UPEC with a GFP encoding plasmid and successfully generated fluorescent UPEC. These fluorescent UPEC were used to infect human bladder epithelial cells (5637) at increasing multiplicities of infection (MOI) to study adherence and invasion. We successfully detected and quantified adherence and invasion of the fluorescent UPEC by different methods that include fluorescent microscopy, flow cytometry, and gentamicin-based invasion assays. Thus, with the assistance of GFP-expressing UPEC, we can efficiently gain more insight on host proteins that mediate adherence and invasion of UPEC. These findings will shed more light on the different mechanisms utilized by UPEC in establishing UTIs, which will in turn lead to the development of more effective therapies for the prevention and treatment of UTIs.

Introduction

Escherichia coli (E. coli) is a Gram-negative, rod-shaped bacteria that is part of the normal flora found in the gastrointestinal tract. It is an opportunist pathogen that can cause diseases in the urinary and gastrointestinal tract (1). There are significant costs associated with the treatment of infections caused by *E. coli* and hence it is vital to understand the pathogenic mechanisms that the bacteria utilizes to infect host cells. An effective way to study the trafficking of bacteria in host cells is by making the bacteria fluorescent.

Green fluorescent protein (GFP) is a commonly used fluorophore that was first isolated from the jellyfish *Aequorea victoria* (2). When the chromatophore inside this protein is excited by blue light it fluoresces by emitting a green light in its excited state. GFP has been used by many researchers to track the movements of proteins, cells or even entire organisms.

Figure 1: The crystal structure of green fluorescent protein (left) isolated from *Aequorea victoria* (right)

The aim of the current study is to generate GFP-expressing UPEC that can be used for studying host-pathogen interactions and trafficking in human bladder epithelial cells. We hypothesize that the interactions between host cells and UPEC can be studied by using fluorescent bacteria. To test this hypothesis, we used 5637 human bladder cells and *E. coli* strain CI5 which is used to study urinary tract infections (3). The findings from this study will enable us to determine if GFP-expressing UPEC can be used to gain more insight into the pathogenic mechanisms employed by the pathogen. These findings will aid researchers in designing better strategies for treating infections.

Results

Bacterial growth after transformation with plasmid encoding GFP

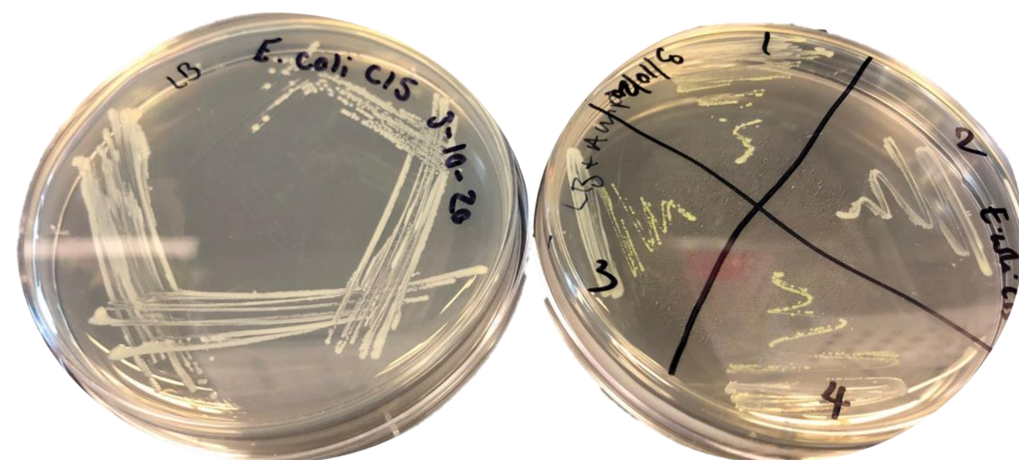
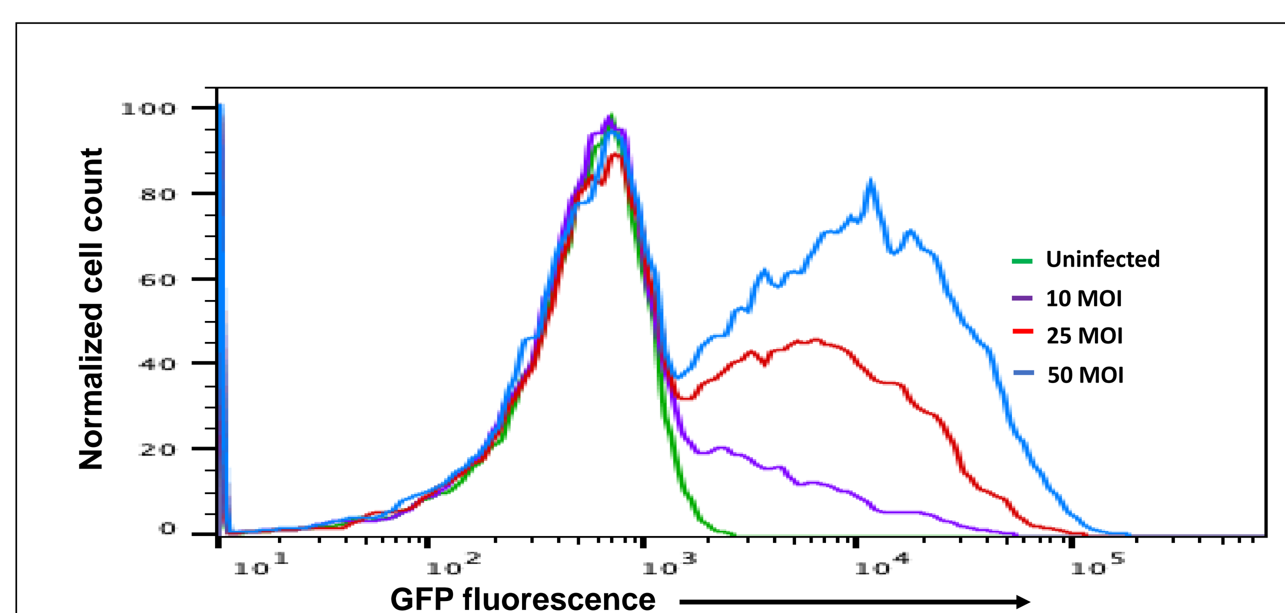


Figure 2: Untransformed *E. coli* CI5 was grown on LB agar (left) while transformed *E. coli* CI5 could grow on LB agar containing ampicillin (right).

Interaction between UPEC and bladder cells increases with MOI



Uninfected | MOI 10 | MOI 25 | MOI 50

1% | 19% | 46% | 57%

Side scatter

GFP fluorescence

Figure 3: 5637 cells were infected with various MOI concentrations of GFP-UPEC and analyzed by flow cytometry. Mean fluorescence of bladder cells at different MOI concentrations (top) and percentage of bladder cells interacting with GFP-UPEC (bottom) is shown.

Interaction of fluorescent UPEC and bladder cells by microscopy

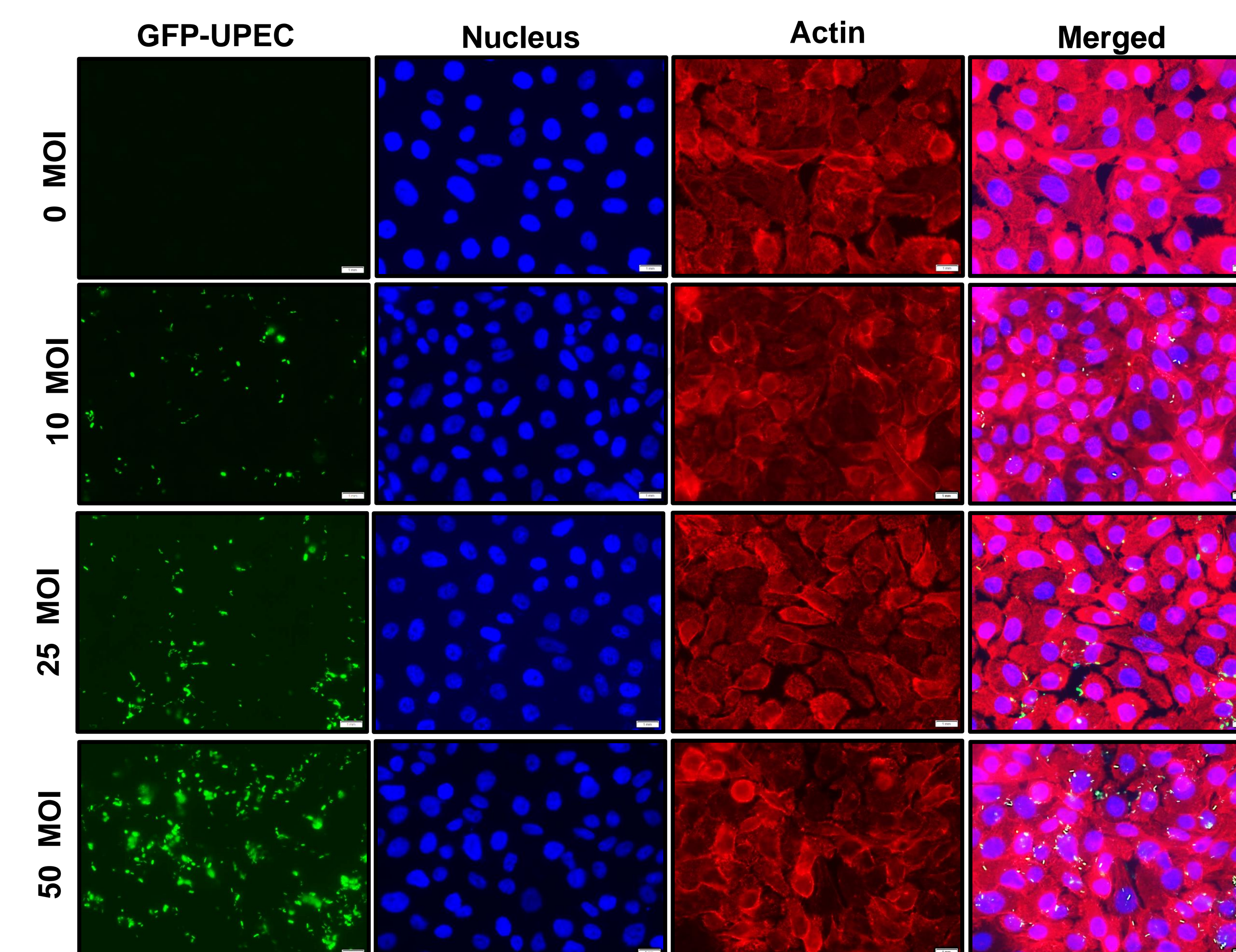


Figure 4: Microscopy analysis of 5637 cells stained with DAPI and phalloidin and infected with GFP-UPEC at increasing multiplicity of infection (MOI) (40X).

Invasion of GFP and non-GFP UPEC in bladder cells

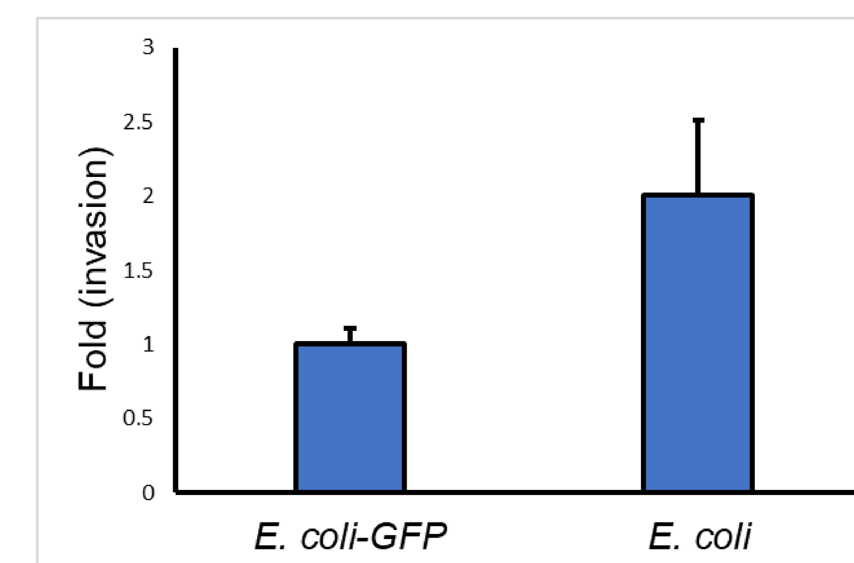


Figure 5: 5637 cells were infected with 25 MOI of GFP expressing UPEC (*E.coli-GFP*) or wild-type UPEC (*E.coli*). Invasion of bacteria was determined by a gentamicin protection assay. Graph shows invasion of wild-type UPEC was ~ 2-fold more than GFP expressing UPEC.

Methods

Transformation of Bacteria

Chemically competent *E. coli* strains were transformed with a plasmid bearing the GFP gene by using the heat shock method. The transformed UPEC were then allowed to grow for one hour in SOC media before plating the bacteria on sterile LB agar plates containing ampicillin.

Flow Cytometry

5637 cells were grown overnight in a 12-well plate. The cells were infected with varying MOIs of fluorescent UPEC and detached using TrypLE. The infected bladder cells were transferred to microcentrifuge tubes on ice and centrifuged at 500 RCF for 5 minutes. The pellet was resuspended, run through a flow cytometer and analyzed using the software FlowJo.

Fluorescent Microscopy

5637 cells were grown overnight on coverslips in a 12-well plate. The cells were infected with varying MOIs of fluorescent UPEC for 1 hour. Cells were washed with 1X PBS, fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and stained with phalloidin. The coverslips were mounted on glass slides using mounting media containing DAPI and viewed under a fluorescent microscope.

Invasion Assay

5637 cells were grown overnight in a 96-well plate. The cells were infected at 25 MOI of fluorescent and non-fluorescent UPEC for 2 hours. Cells were washed then treated with gentamicin for 1 hour. The cells were lysed and plated on sterile LB plates at 1:5 dilution. Graph was created using Microsoft Excel based on CFU enumeration.

Discussion

Based on our results, we were able to observe interactions of GFP expressing UPEC with host bladder cells, thereby supporting our hypothesis. We transformed a plasmid encoding the GFP gene into the *E. coli* CI5 strain and performed fluorescent microscopy and flow cytometry experiments, which confirmed interactions between the fluorescent UPEC and the 5637 cells. Gentamicin-based invasion assays showed that our fluorescent bacteria exhibits invasion in bladder cells though it is not as invasive as the wild type. Thus, fluorescent bacteria can be used in future experiments to understand the pathogenic mechanisms as well as trafficking of UPEC in host cells. These studies will further our understanding on host-pathogen interactions that will enable us in the design of better therapeutics to treat infections caused by *E. coli.*

References & Acknowledgements

1. Kaper JB, Nataro JP, & Mobley HLT. (2004). Nature Reviews Microbiology, Vol. 2, 123-140.
2. Chalfie M, Tu Y, Euskirchen G, Ward WW & Prasher DC. (1994). Science, Vol. 263, 802-5.
3. Mehershahi KS, Abraham SN, Chen SL. (2015) . Genome Announcements, Vol. 3, pii: e00558-15.

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