Glowing Bacteria: A Way to Study Host-Pathogen Interactions

Alissa D. Eberhard and Janaki K. Iyer
Gregg Wadley College of Science and Health Professions, Northeastern State University

Abstract

Escherichia coli (*E. coli*) is a gram-negative, motile bacterium that is abundantly found in our gastrointestinal tract. They are key to a healthy digestive system in humans and animals. However, they are opportunistic pathogens. Some strains of *E. coli* are pathogenic and can cause gastrointestinal as well as urinary tract infections (UTIs). They are the causative agent for over 75% of UTIs. The virulence factors expressed by some strains of this bacterium, such as adhesins and fimbriae, allow the pathogenic bacteria to adhere and invade the host cells. UTIs caused by these invasive uropathogenic *E. coli* are difficult to treat. To gain a better understanding of pathogenic mechanisms used by invasive *E. coli*, we want to study the differences in cellular trafficking and colonization patterns between non-pathogenic and pathogenic strains of *E. coli*. The nonpathogenic strain used in this study is *E. coli* K12 while the pathogenic strain is *E. coli* CI5 which causes UTIs. To visualize differences in cellular trafficking between the two strains of *E. coli* in host cells, the bacteria were made chemically competent by the calcium chloride method. A plasmid expressing green fluorescent protein (GFP) was introduced into the bacteria by the heat shock method of transformation. The success of plasmid transformation in both bacterial strains was determined by plasmid isolation followed by digestion with restriction enzymes. Expression of GFP was confirmed by fluorescent microscopy to visualize the presence of fluorescent bacteria. These fluorescent strains of bacteria will aid us in understanding the differences in adhesion and invasion between the non-pathogenic and pathogenic strains of *E. coli* in host cells and thereby help us in designing new therapies to treat UTIs caused by invasive *E. coli*.

Introduction

Escherichia coli (*E. coli*) is a Gram-negative, rod-shaped bacteria that is commonly 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). The amino acid residue inside this protein is a chromatophore that when excited by a blue light emits a green light in its excited state. GFP has been used by many researchers to track the movements of proteins, cells or even bacteria.

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

The aim of the current study is to introduce the gene encoding for GFP in a pathogenic and non-pathogenic strain of *E. coli. E. coli* strain K12 is a non-pathogenic strain that can be used as control for experiments involving pathogenic strains of *E. coli*. *E. coli* strain CI5 is a pathogenic strain that is used to study urinary tract infections (3). The resulting fluorescent bacteria will be used for studies to gain more insight into the trafficking of pathogenic bacteria in host cells. These findings will aid researchers in designing better strategies for treating infections caused by *E. coli*.

Results

Bacterial growth was observed in selection plates after transformation with plasmid encoding GFP

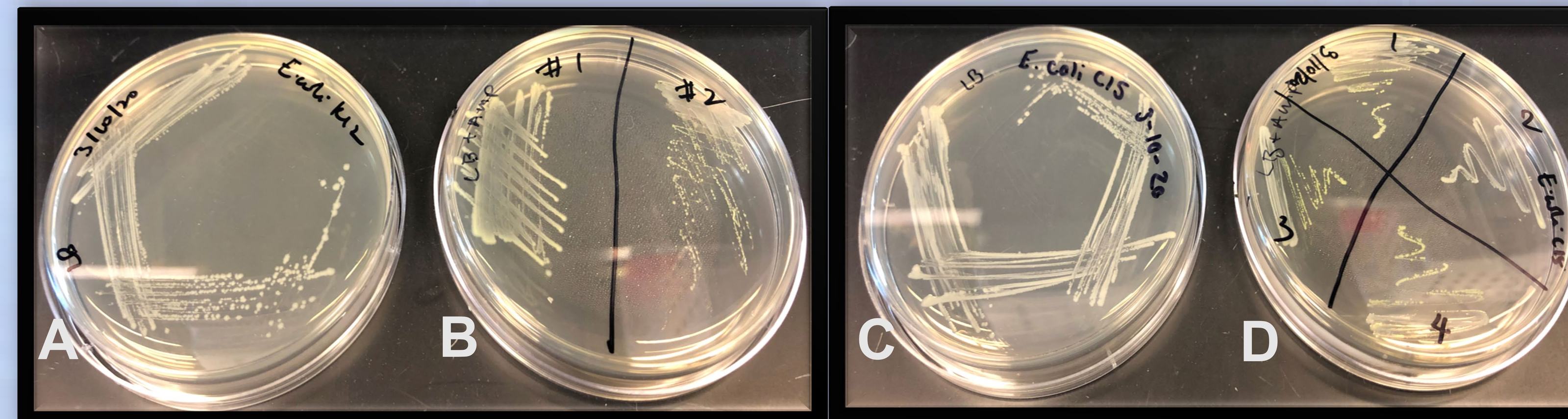


Figure 2: Untransformed *E. coli* K12 was grown on LB agar (A) while transformed *E. coli* K12 could grow on LB agar containing ampicillin (B). Untransformed *E. coli* CI5 was grown on LB agar (C) while transformed *E. coli* CI5 could grow on LB agar containing ampicillin (D).

Transformed *E. coli* showed the presence of plasmid

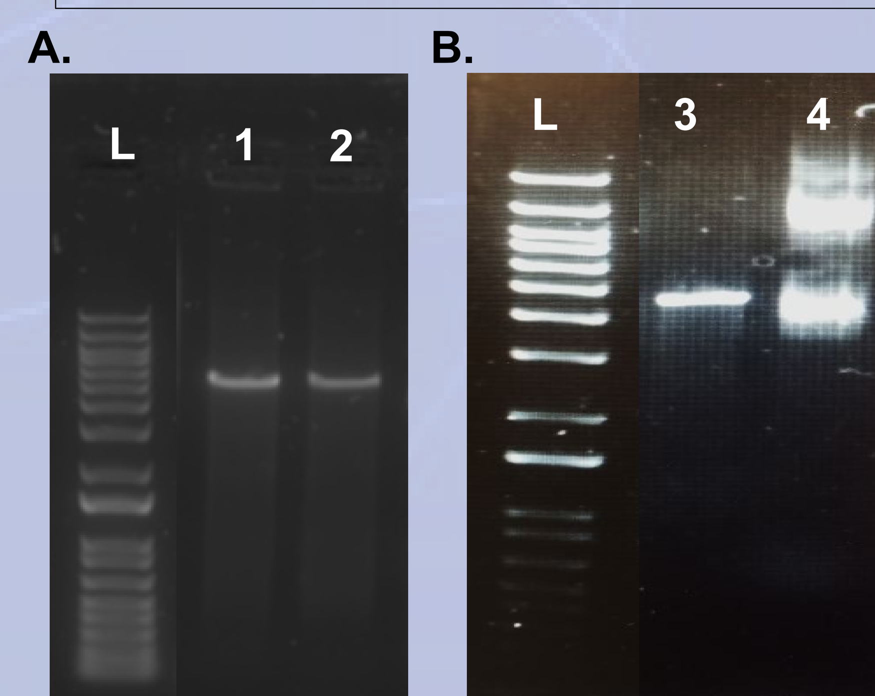


Figure 3: Plasmid from transformed E. coli K12 (A) or E. coli CI5 (B) was isolated using the alkaline lysis method and run on a 0.8% agarose gel after restriction digestion. The loading for gels is L: ladder, 1: digested plasmid from *E. coli* K12 (clone 1); 2: digested plasmid from *E. coli* K12 (clone 2); 3: digested plasmid from *E. coli* CI5, 4: undigested plasmid from *E. coli* CI5

Transformed *E. coli* strains are fluorescent

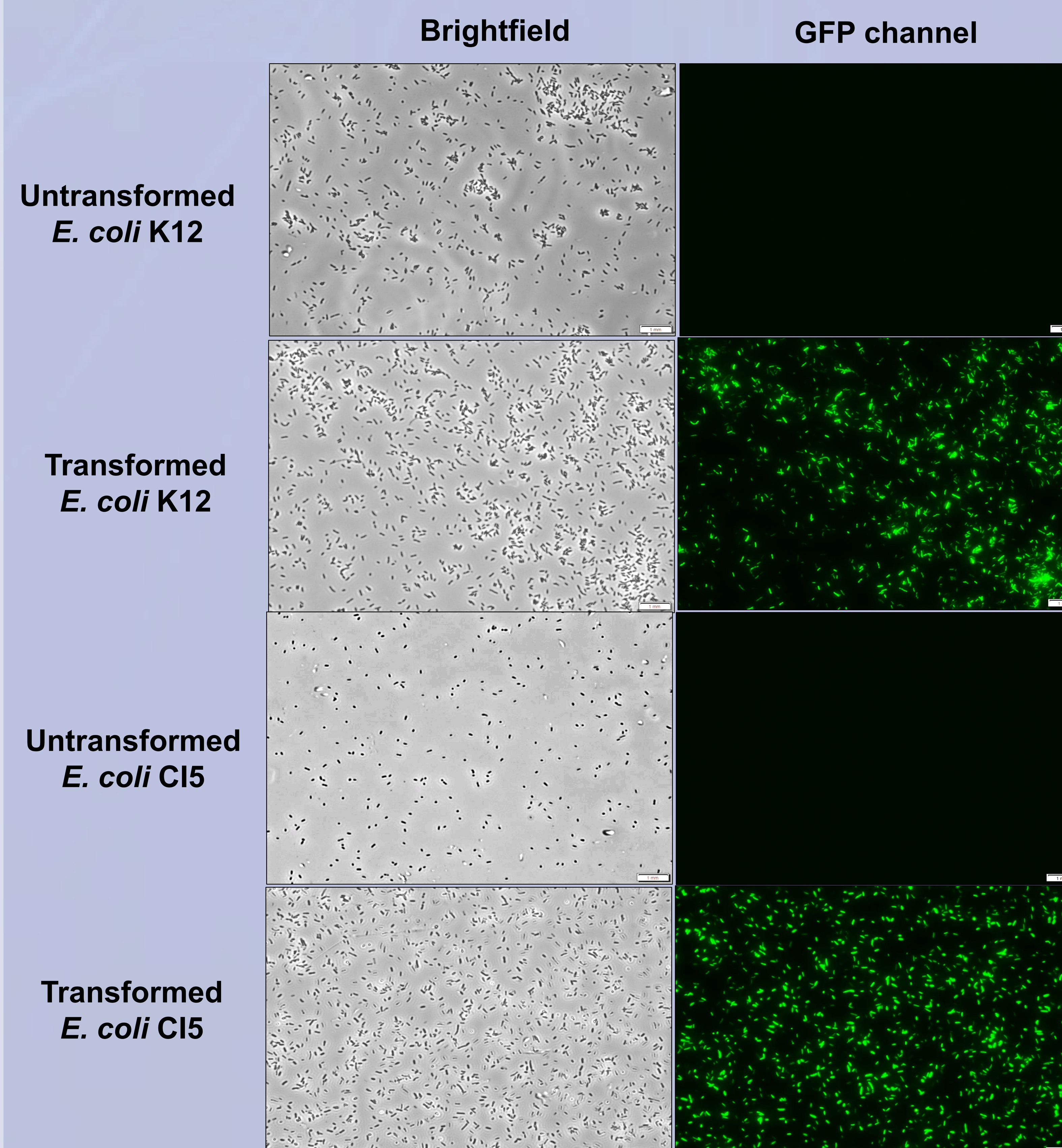
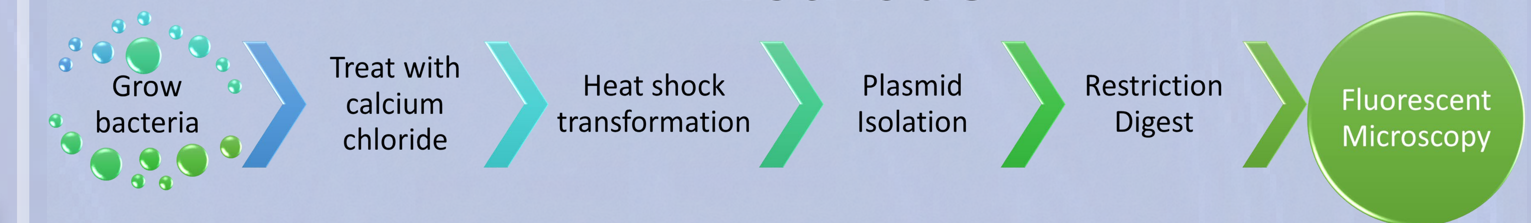


Figure 4: Microscopy analysis of *E. coli* strains to detect GFP fluorescence (40X).

Methods



Preparation of chemically competent bacteria

E. coli K12 and *E. coli* CI5 were made chemically competent using calcium chloride. Briefly, bacteria were grown in LB broth till the bacteria were in exponential phase of growth. After harvesting, the bacteria were treated with 100 mM of sterile cold calcium chloride solution to make the bacteria competent. The competent bacteria were frozen and stored at -80°C until further use.

Transformation of bacteria

Chemically competent *E. coli* strains were transformed with a plasmid bearing the GFP gene by using the heat shock method. Briefly, the bacteria were incubated with the plasmid at 4°C followed by heating the bacteria for 45 seconds at 42°C. The bacteria were then allowed to grow for one hour in SOC media before plating the bacteria in sterile LB agar plates with ampicillin.

Plasmid Isolation and Restriction Digestion

Plasmid was isolated from transformed *E. coli* strains using the Qiagen miniprep kit according to the manufacturer's instructions. The principle of the kit is based on the alkaline lysis method. Isolated plasmid was digested with EcoRI and ran on a 0.8% agarose gel by gel electrophoresis.

Fluorescent Microscopy

GFP expression in transformed bacteria was confirmed by fluorescence microscopy.

Discussion

Based on our results, we were successful in transforming a plasmid encoding the GFP gene into *E. coli* K12 and *E. coli* CI5 strains. We were able to selectively grow the transformants in LB agar plates containing the selection antibiotic, ampicillin. GFP expression was confirmed by fluorescent microscopy since only the transformed bacteria were able to fluoresce as observed in the FITC channel. These fluorescent bacteria can be used in future experiments to understand the pathogenesis mechanisms as well as trafficking on pathogenic and non-pathogenic *E. coli* in host cells. These studies will further our understanding on host-pathogen interactions that will in turn enable in the design of better therapeutics to treat infection caused by *E. coli.*

References

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