Novel fluorescent reporters for studying pathogen-host interactions
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Introduction

Fluorescent proteins, such as green fluorescent protein (GFP), have diverse applications in the basic and applied sciences. While GFP has been frequently used in eukaryotic systems, its applications have been limited in microorganisms due to a lack of broad-range molecular tools. In this study, we have developed a vector to express GFP in pathogenic bacteria for use in bacterial pathogenesis and pathogen-host studies. A shuttle vector encoding the GFP variant mCherry (pUCP18-MCSgfpmut3) was generated and successfully transformed into various Gram-negative opportunistic pathogens from the ATCC collection, including: Escherichia coli (ATCC 25922™), Salmonella enterica (ATCC 14028™), Shigella flexneri (ATCC 12022™), Pseudomonas aeruginosa (ATCC 10145™), and the P. aeruginosa type strain PA01 (ATCC 15692™). P. aeruginosa was used as a model to test the characters of the vector and sensitivity of detection using a fluorescence plate reader, microscopy, flow cytometry, and in vivo imaging systems.

Results

The expression of the gfpmut3 gene was monitored during growth (Figure 1). GFPmut3 did not alter bacterial growth in either P. aeruginosa (Figure 1A) or E. coli (data not shown). Fluorescence was easily detected and quantified using a fluorescence microplate reader (Figure 1B). A linear correlation was observed between fluorescence and colony forming units (CFU) (R²=0.999) or optical density (OD) at 600 nm concentrations ranging from 10⁶ to 10⁹ CFU/mL (Figure 1C).

Figure 1: Expression of GFPmut3 does not affect bacterial fitness and can be used for bacterial quantification.