Application of dimaleimide fluorogen tagging strategy to study bacterial physiology

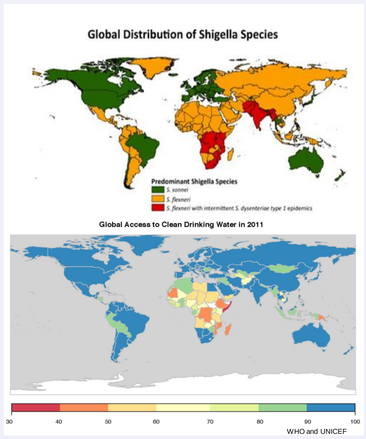
Agnes Cadieux, Francois-Xavier Campbell-Valois, Host-Microbe Interaction Laboratory, University of Ottawa

Abstract

Little is known about the pathogenesis of the gastrointestinal pathogen *Shigella flexneri*. The virulence abilities of this organism to penetrate host epithelial cells and transfer its contents via the Type Three Secretion System are relatively unknown. In order to determine the pathway, surface, periplasmic, and cytoplasmic plasmids are tagged with a short peptide sequence consisting of two Cys residues which can react with a dimaleimide fluorogen. In order to determine the fitness of the bacteria with the newly tagged plasmids, an avirulent strain of electrocompetent *E. coli* is transformed, grown on simple media, and sequenced.

Introduction

***Shigella* species are a causative agent of a bacterial dysentery in humans known as shigellosis. It causes 5-15% of all diarrhea worldwide, and 0.5-1 million deaths per year, two thirds of which occur in children <5 years of age. They are Gram-negative, intracellular aerobes. Infections are localized to the outermost layer of the intestinal wall of the colon, and invasion of tissues by the organism results in a strong inflammatory reaction that destroys the epithelium. Infectious dose can be as little as 10 organisms, and mode of transmission is by fecal-oral route.**



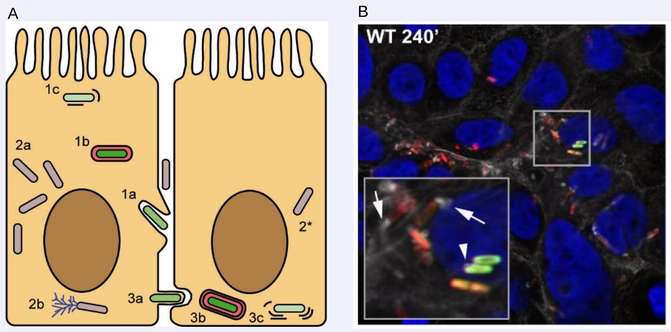


Figure 1A) Infectious process of *Shigella* within a host epithelial cell. (1a) internalization, (1b) bacteria within phagosome, (1c) destruction of phagosome by bacterial enzymes, (2a) replication within cytosol, (2b) recruitment of host actin filaments for production of comet tails, (3a) reactivation of T3SS and formation of protrusions into adjacent cell, (3b) bacteria within double membranous vacuole (3c) destruction of vacuole by bacterial enzymes, (2*) cycle repeats. 1B) Confocal microscopy image of cell-to-cell spread. Arrowheads point to active bacteria, arrows are pointing to inactive bacteria.

***Shigella* uses a type III secretion system to inject effector proteins into host cells. However, detecting and monitoring of the T3SS of intracellular bacteria is challenging. Previous studies have shown T3SS activity upregulated during initial bacteria entry, downregulated within the cytoplasm, and reactivated during contact with the plasma membrane of an adjacent cell, promoting cell-to-cell spread.**

Rationale

A fluorescent protein-labeling strategy was developed by the Keillor lab where a protein of interest is genetically tagged with a short peptide sequence presenting two Cys residues that can selectively react with synthetic fluorogenic reagents. The sequence, called DC10* was inserted into three plasmids of interest, for surface expression, periplasmic expression, and cytoplasmic expression.

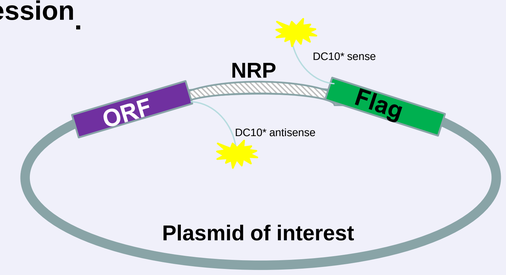


Figure 2) Scheme of DC10* insertion into plasmid. ORF: open reading frame; NRP: non-reacting peptide. Flag: flag tag already present in plasmid.

Once primers are inserted and transcribed, we can follow the response through to secretion, allowing us to understand when the bacterium is sending out effectors into the host cell and continuing the infectious process.

Results

Figure 3) LB agar with growth of electrocompetent *E. coli* harboring the ligated cytoplasmic plasmid. 30µg/mL of chloramphenicol was used as the sterility control.

After the plasmids were amplified by PCR, they were purified using the GENJet plasmid kit. Yield was confirmed on agarose gel, and the plasmids were phosphorylated, and ligated overnight at room temperature. Electrocompetent cells were made using a gene pulse controller. Cells were then plated on LB agar infused with either ampicillin or chloramphenicol to match the resistance gene of the corresponding plasmid. Plates were incubated overnight at 37°C. Figure 3 shows successful transformation of the cytoplasmic plasmid into electrocompetent *E. coli*.

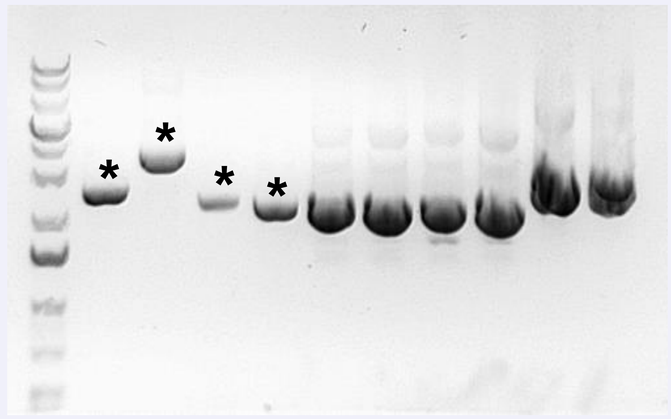


Figure 4) Protein Electrophoresis on agarose gel of five PCR products run in duplicate. Order of loading are: MW ladder, surface control (pMK90), surface (pMK90), cytoplasmic (Mbp), periplasmic (Mbpp), effector (pUC18)).

In order to confirm isolation of the plasmids after transformation with the electrocompetent *E. coli*, protein electrophoresis was run on an agarose gel post Miniprep procedure. In this step all bacterial DNA is removed, leaving the plasmid free for isolation, quantification, and sequencing. In figure 4 the thick, rounded bands of the cytoplasmic, periplasmic, and effector plasmids speak to excellent isolation of supercoiled DNA product. However, the lower molecular weight bands observed in the wells identified by stars are false positives. This may be explained by toxicity often observed with surface-expressing plasmids in bacteria.

Future Direction

Sequence analysis was carried out by an external source and proved successful for the cytoplasmic, periplasmic and effector plasmids. Expression of the recombinant proteins will be confirmed via western blot, and the reactivity of the fluorescent dimaleimide compound will be tested against the tagged proteins. The experiment will then be repeated using *S. flexneri*. If successful, the tagged colonies will be incubated with tissue culture cells and observed via real-time fluorescence during the infectious process.

References

Buchrieser, Carmen, Philippe Glaser, Christophe Rusniok, Hafed Nedjari, Helene D'hauteville, Frank Kunst, Philippe Sansonetti, and Claude Parsot. "The Virulence Plasmid PWR100 and the Repertoire of Proteins Secreted by the Type III Secretion Apparatus of Shigella Flexneri." *Molecular Microbiology Mol Microbiol* 38.4 (2000): 760-71.

Campbell-Valois, François-Xavier, Pamela Schnupf, Giulia Nigro, Martin Sachse, Philippe J. Sansonetti, and Claude Parsot. "A Fluorescent Reporter Reveals On/Off Regulation of the Shigella Type III Secretion Apparatus during Entry and Cell-to-Cell Spread." *Cell Host & Microbe* 15.2 (2014): 177-89.

Chen, Yingche, Christopher M. Clouthier, Kelvin Tsao, Miroslava Strmiskova, Hugo Lachance, and Jeffrey W. Keillor. "Coumarin-Based Fluorogenic Probes for No-Wash Protein Labeling." *Angewandte Chemie Angew. Chem.* 126.50 (2014): 14005-4008.

Public Health Agency of Canada. Pathogen Safety Data Sheet. Shigella spp. (2015). http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/shigella-eng.php

Acknowledgements

I would like to thank Dr. Francois-Xavier Campbell-Valois, Dr. Jeffrey W. Keillor, and the Marion lab biochemistry group.