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The Effects of host physiological conditions on the expression of icsP in *Shigella flexneri*

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THE EFFECTS OF HOST PHYSIOLOGICAL CONDITIONS ON THE EXPRESSION OF ICSP IN *SHIGELLA FLEXNERI*

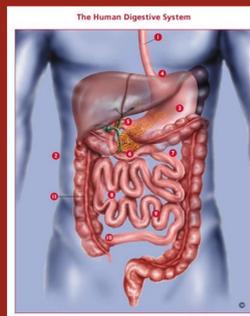
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Poster Presentation

Shigella flexneri is a gram-negative bacterium capable of causing diarrhea and dysentery known as shigellosis. It is estimated there are 167.4 million shigellosis episodes throughout the world each year causing 1.1 million deaths. *Shigella* invades cells in the lower intestine through an induced phagocytosis. Once in the cytoplasm, bacteria move from one cell to another using actin-based motility. The *Shigella* outer membrane protease IcsP regulates actin-based motility and cell-to-cell spread by cleaving the actin assembly protein IcsA from the bacterial cell surface. We hypothesize that IcsP may serve additional functions during infection. By examining which environmental signals trigger *icsP* expression, we aim to identify other regions of the body where IcsP might function. *Shigellae* are exposed to an array of environmental conditions in the body. We examined the presence of bile salts, low pH, and anaerobic conditions in this study. Expression of *icsP* and IcsP levels were assessed in bacteria grown under each of these physiological conditions using β -galactosidase assays and western blots, respectively. Growth of *Shigella* strains was reduced in the presence of deoxycholate, a common bile salt, as compared to the control. In stationary growth phase, *icsP* expression increased when the bacteria were grown in the presence of bile salts. Growth of *Shigella* in medium buffered at pH 5.5 was slightly elevated (<10% more growth) when compared to bacteria grown in medium at pH 7.4. We also found that anaerobic conditions negatively impact the growth of *Shigella*. Expression of *icsP* has not yet been measured under this condition.

Introduction

Shigella flexneri is a gram-negative bacterium that is capable of invading intestinal epithelial cells, causing a severe diarrhea and dysentery known as Shigellosis (LaBrec *et al.*, 1964). The annual number of Shigellosis episodes throughout the world is estimated to be 164.7 million with 1.1 million deaths in developing countries (Kotloff, 1999). The bacteria infect the host through an induced phagocytosis (Clerc, 1987). They move through the cytoplasm of host cells and are able to invade adjacent cells through actin-based motility (Shere, 1997). *IcsA* is a unipolar protein on the surface of the bacterium that is required for actin-based motility (Shere, 1997). The protease *IcsP* is able to cleave *IcsA* from the bacterial surface, but the precise role of *IcsP* in the overall virulence of *Shigella* is not fully understood (Steinhauer, 1999).

During the course of an initial infection, *Shigellae* are exposed to an array of environmental conditions as they move through the various systems of the body, and they must be able to survive in these conditions to produce a viable infection.



3. The Stomach

- Low pH

6.-11. Small Intestine, Large Intestine, and Colon

- High Osmolarity
- Bile Salts Present in Small Intestine
- Anaerobic
- Low Iron in Host Epithelial Cells

Figure 1: Potential physiological challenges *Shigellae* face during an initial infection.

Materials and Methods

Strains of *Shigella flexneri*

- Serotype 2a
- Wild-type: 2457T (LaBrec *et al.* 1964)
- virB* mutant: AWY3 (Wing *et al.* 2004)

Growth Curves

- Used to identify exponential and stationary phases of growth

Western Blots

- Used to quantify *IcsP* production
- Total cell proteins were normalized to cell density
- Tests post-transcriptional regulation of *IcsP*

B-galactosidase Assays

- Used to assess activity of the *icsP* promoter
- Tests transcriptional regulation of *icsP*

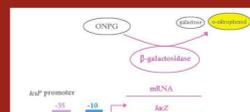


Figure 2: The *lacZ* gene is under the control of the *icsP* promoter. β -galactosidase cleaves ONPG into galactose and o-nitrophenol.

Aim Of This Study

The aforementioned environmental factors can potentially have dramatic effects on the global gene expression of the organism, allowing the bacterium to adapt to these intra-host environments. This study examines what effect(s), if any, these environmental conditions may have on the expression of *icsP* and production of the corresponding protease.

Results: Low pH

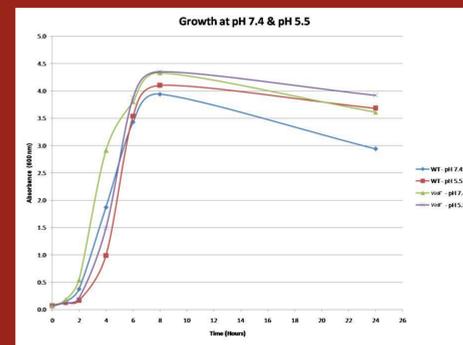


Figure 3: WT and the *virB* mutant were grown under acidic conditions using MES buffer at pH 5.5 and under neutral pH using MOPS buffer at pH 7.4. Acidic conditions did not significantly effect overall growth of either *Shigella* strain or their entry into particular phases of growth.

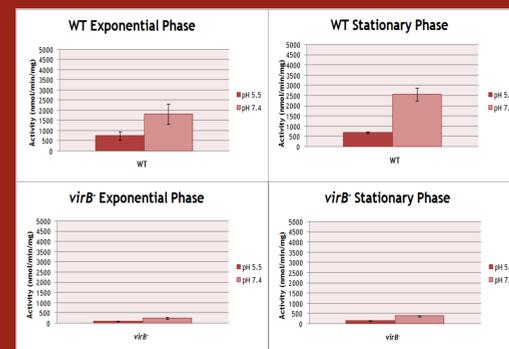


Figure 4: β -galactosidase assays were used to assess *icsP* promoter activity under neutral pH and acidic conditions. *icsP* expression was increased during growth at pH 7.4 compared to pH 5.5 in both exponential and stationary growth phases. Promoter activity was 10-fold higher in WT than in the *virB* mutant. This was expected because *VirB* is known to positively regulate the *icsP* promoter.

Results: Bile Salts

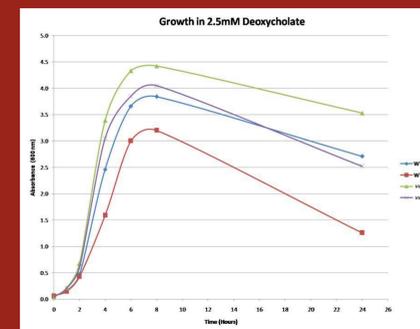


Figure 5: WT and the *virB* mutant were grown in LB or LB supplemented with 2.5mM deoxycholate, a common bile salt. Growth of both strains was decreased by 25% in the presence of deoxycholate; however, bile salts did not effect entry into exponential or stationary phases of growth.

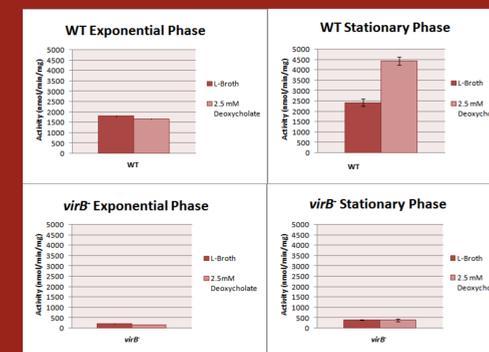


Figure 6: β -galactosidase assays were used to assess *icsP* promoter activity in the presence of bile salts. The activity of the *icsP* promoter was increased in the WT strain only in the stationary phase of growth.

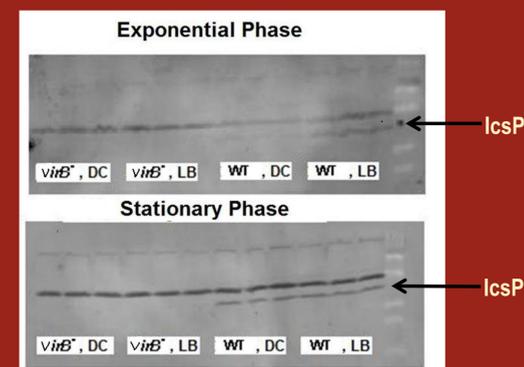


Figure 7: Western blots were used to examine how much *IcsP* was present in the cells. Overall levels of *IcsP* were higher in cells harvested during stationary phase than in exponential phase, and cells grown in LB during exponential phase showed higher levels of *IcsP* compared to those grown in deoxycholate.

Results: Anaerobic Conditions

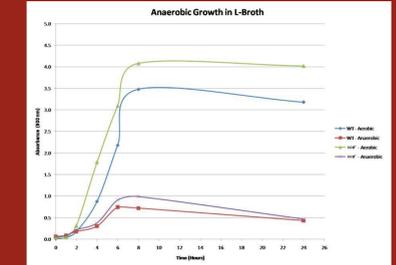


Figure 8: WT and the *virB* mutant were grown in anaerobic conditions which were created by sparging each sample with nitrogen. It was shown that the overall growth of *Shigella* is negatively impacted by the absence of oxygen. *icsP* expression has not yet been assessed under this condition.

Conclusions/Future Directions

IcsP protein levels and *icsP* promoter activity both increase when *Shigella* cultures enter stationary phase

- Future experiments will explore the possibility that *icsP* expression is dependent on cell density

Control at the level of transcription is the most important regulatory step

- icsP* promoter is known to be regulated by H-NS, a histone-like repressor protein that blocks transcription at the *icsP* promoter

- Increased activity at the *icsP* promoter in bile salts may be due to H-NS dissociating from the promoter

- Future experiments using an *hns* mutant in the presence of deoxycholate will test this hypothesis

Growth is significantly decreased in anaerobic conditions

- We propose that the *Shigellae* are not growing to higher cell densities because they are fermenting

- In future experiments, an alternative electron acceptor, such as NO_3^- , will be provided to increase growth of the culture. We can then determine whether the absence of oxygen influences *icsP* promoter activity.

Acknowledgements

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Works Cited

- Clerc, P.L., et al., Plasmid-mediated early killing of eucaryotic cells by *Shigella flexneri* as studied by infection of J774 macrophages. *Infect Immun*, 1987, 55(3): p. 521-7.
- Kotloff, K.L., et al., Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ*, 1999, 77(8): p. 651-66.
- LaBrec, E.H., et al., Epithelial Cell Penetration as an Essential Step in the Pathogenesis of Bacillary Dysentery. *J Bacteriol*, 1964, 88(5): p. 1503-18.
- Shere, K.D., et al., Disruption of *IcsP*, the major *Shigella* protease that cleaves *IcsA*, accelerates actin-based motility. *Mol Microbiol*, 1997, 25(3): p. 451-62.
- Steinhauer, J., et al., The unipolar *Shigella* surface protein *IcsA* is targeted directly to the bacterial old pole: *IcsP* cleavage of *IcsA* occurs over the entire bacterial surface. *Mol Microbiol*, 1999, 32(2): p. 367-77.