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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.
The Effects of Host Physiological Conditions on the Expression of icsP in Shigella flexneri

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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 worldwide 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 (Gilles, 1987). They move through the cytoplasm of host cells and are able to invade adjacent cells through actin-based motility (Shane, 1997) IcsA is a unipolar protein on the surface of the bacterium that is required for actin-based motility (Shane, 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 (Steinhauser, 1999).

During the course of an initial infection, Shigellas 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.

Materials and Methods

• Strains of Shigella flexneri
  • Serotype 2a
  • Wild-type: 2457T (LaBrec et al., 1964)
  • icsP 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 normalized to cell density
  • Tests post-transcriptional regulation of IcsP

• β-galactosidase Assays
  • Used to assess activity of the icsP promoter
  • Tests transcriptional regulation of icsP

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

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

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 affect overall growth of either Shigella strain or their entry into particular phases of growth.

Results: Low pH

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.

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.

Results: Bile Salts

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 hnr+ mutant in the presence of deoxycholate will test this hypothesis

• Growth is significantly decreased in anaerobic conditions
  • We propose that the Shigellas are not growing to higher cell densities because they are fermenting
  • In future experiments, an alternative electron acceptor, such as NO₃-, will be provided to increase growth of the culture. We can then determine whether the absence of oxygen influences icsP promoter activity.

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Works Cited