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Regulation of the Shigella flexneri icsP gene and H-NS dependent repression

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Introduction

Shigella flexneri is a gram negative bacterium which is known to cause dysentery in humans and primates. *S. flexneri* is the cause of over a million deaths worldwide especially in children and the elderly. When Shigella enters its host it is able to recruit actin from the host's cell, forming an actin based tail that allows it to move. The outer membrane protein which recruits actin is IcsA, and the protein which regulates IcsA is IcsP, where IcsP cleaves excess IcsA. The *icsP* gene is regulated by VirB and the histone-like nucleoid structuring protein (H-NS) which are two transcription factors. At 30°C H-NS represses transcription of virulence genes and at 37°C VirB derepresses virulence genes. Our goal is to identify the DNA sequences required for the H-NS dependent repression of the *icsP* promoter. These studies will improve our understanding of the regulation of the *icsP* promoter, and this knowledge may be helpful when studying the regulation of other virulence genes in *S. flexneri*.

Previous studies tell us H-NS dependent repression of the *icsP* promoter requires sequences located between -893 and -351 with respect to the transcription start site (TSS) (Fig. 1 & 2)

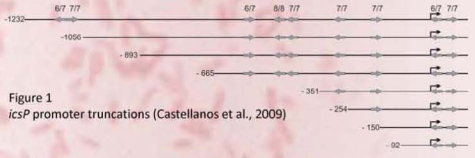

Figure 1
icsP promoter truncations (Castellanos et al., 2009)

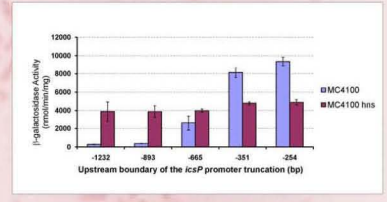

Figure 2
Experiment to identify regions of the *icsP* promoter required for H-NS dependent repression

Hypothesis 1

H-NS dependent repression requires sequences between -637 and -351 with respect to TSS.

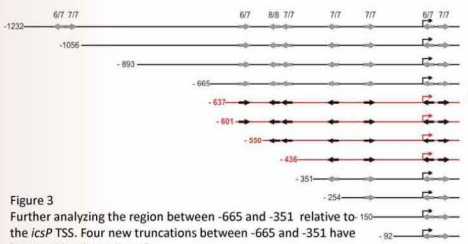

Figure 3
Further analyzing the region between -665 and -351 relative to the *icsP* TSS. Four new truncations between -665 and -351 have been made, shown in red

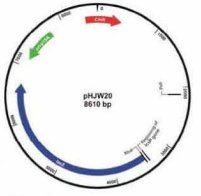

Figure 4
Promoter fragments were cloned into pHJW20 to measure Beta Galactosidase activity.

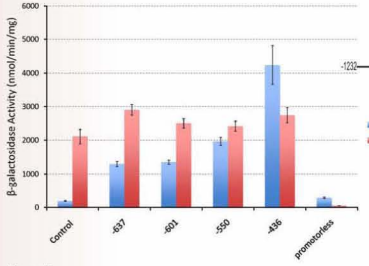

Figure 5
icsP promoter activity is less repressed near the -436 truncation

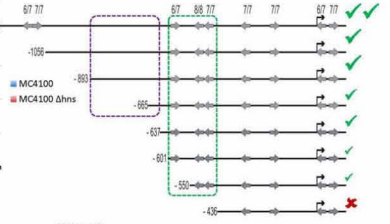

Figure 6
Sequences -893 to -637 and -601 to -436 are required for H-NS dependent repression

My second goal is to further characterize the *PicsP-lacZ* reporter plasmid. Specifically we want to know if *icsP* promoter activity is being affected by an upstream gentamycin cassette.

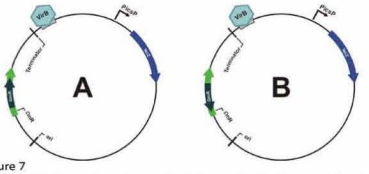

Figure 7
In plasmid A the gentamycin cassette is transcribed in the same direction as P*icsP*. In plasmid B the gentamycin cassette is transcribed in the opposite direction as P*icsP*.

Hypothesis 2

The gentamycin cassette is affecting *icsP* promoter activity.

Material and Methods

To test this we will insert a transcription terminator after the gentamycin cassette stopping all transcription before the *icsP* promoter.

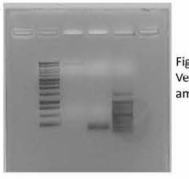

Figure 8
Vector for terminator (8573 bps) and insert (241 bps) amplification by PCR

Conclusion

1. H-NS dependent repression of the *icsP* promoter requires two sequences between -893 to -637 and -601 and -436.
2. Insert lambda *oop* (transcription terminator) was unable to be amplified by PCR, possible hairpin occurring.

Future Directions

1. Zone in on the two sequences between -893 to -637 and -601 to -436 to determine if H-NS binds within these regions.
2. Further analyze regions -893 to -637 and -601 to -436 using protein experiments
3. Assay these truncations in the presence of VirB
4. Instead of PCR amplification of transcription terminator, we will isolate the terminator DNA from a plasmid using restriction enzymes.

Acknowledgements

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