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The Regulation of the ospZ and ORF-2 promoters in the Shigella flexneri by the virulence factor VirB

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Shigella flexneri is a pathogenic bacterium that is the causative agent of shigellosis, an illness characterized by severe dysentery. Shigella carries many of its virulence genes on a large virulence plasmid and consequently this plasmid is the focus of research in the Wing lab. My research focuses on the transcriptional regulation of a newly identified gene called ospZ. This gene’s protein product is secreted outside the bacterial cell and assists in polymorphonuclear leukocyte migration, a function that is believed to enhance the virulence of Shigella. Many genes encoded by the Shigella virulence plasmid are regulated by the transcription factor VirB, which is also encoded by the virulence plasmid. VirB regulates the expression of IcsP, a gene 1.6 kilobase pairs upstream of ospZ on the divergent strand. To determine the role VirB plays in the regulation of ospZ, reporter plasmids will be constructed in which the ospZ promoter region is fused to lacZ (a gene that encodes the enzyme beta-galactosidase) and transformed into wild type and VirB mutant strains of Shigella. Promoter activity of ospZ will then be measured using beta-galactosidase assays. My hypothesis is that VirB, which binds to DNA 100 base pairs upstream of the ospZ gene, regulates the expression of this gene also.
The Regulation of the *ospZ* and ORF-2 Promoters in *Shigella flexneri* by the Virulence Factor VirB

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**Introduction**

*Shigella flexneri* is a pathogenic bacterium that is the causative agent of shigellosis, an illness characterized by severe dysentery. My research focuses on the transcriptional regulation of a newly identified gene on the *Shigella* virulence plasmid called *ospZ*, and a hypothetical gene immediately upstream of *ospZ* called ORF-2. The *ospZ* gene product is secreted outside the bacterial cell and assists in polymorphonuclear leukocyte migration, a function that is believed to enhance the virulence of *Shigella* [1]. Many genes encoded by the *Shigella* virulence plasmid are regulated by the transcription factor VirB, which is also encoded by the virulence plasmid. VirB regulates the expression of *icsP*, a gene 1.6 kilobase pairs upstream of *ospZ* on the divergent strand. My hypothesis is that VirB, which binds to DNA 200 base pairs upstream of *ospZ* and ORF-2, regulates the expression of these genes as well.

**Objectives**

- Construct *lacZ* reporter plasmids containing the putative promoter regions of *ospZ* and ORF-2.
- Conduct β-galactosidase assays with the newly constructed plasmids to measure promoter activity of *ospZ* and ORF-2 in wild type and *virB* mutant strains of *Shigella flexneri*.

**Materials and Methods**

**Plasmid Constructs:**

- 1651bp and 1430bp promoter regions upstream of *ospZ* and ORF-2, respectively, were PCR amplified from the virulence plasmid (Fig.1) and cut with *Sau*II and *Xba*I. The vector (pHJW20), carrying the *lacZ* gene and chloramphenicol resistance, was cut with the same restriction enzymes and ligated to the promoter inserts to create pDB05 (Fig.2) and pDB02 (Fig.3). These plasmids, along with pMIC21, were then transformed into wild type and *virB* mutant strains of *Shigella*.

**Results**

- **Wild type Shigella flexneri AWY3**: *virB* mutant *Shigella flexneri pDB05: ospZ* promoter fused to pHJW20
- **pDB02: ORF-2 promoter fused to pHJW20**
- **pMIC21**: Promoterless *lacZ* construct serving as negative control

**β-galactosidase Assays:**

The activities of the promoters fused to *lacZ* were indirectly measured. *lacZ* encodes β-galactosidase, an enzyme that cleaves a colorless substrate (ONPG) to form a yellow product, the amount of which can be measured using spectrophotometry. The level of absorbance is directly related to the amount of enzyme present, which in turn is a measurement of promoter activity.

The results indicate that the ORF-2 promoter has very low activity regardless of the presence or absence of VirB.

**Conclusion**

- ORF-2 does not have a promoter
- The *ospZ* promoter must contain sequence within ORF-2

**Future Directions**

- Identification of the transcription start site of *ospZ*

**References**


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