Characterization of *rfbB* as a virulence-associated gene in *B. cepacia*

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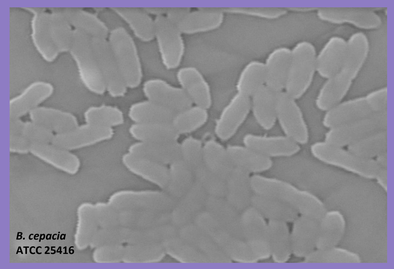


Abstract

Burkholderia cepacia is a gram-negative bacterium that is a known pathogen of plants and humans. *B. cepacia* was first described as the causative agent of sour skin rot in onion crops and has more recently been recognized as an opportunistic pathogen in cystic fibrosis patients where it is able to colonize the airway and cause rapid pulmonary decline. There are limited treatments available for this bacterium due to its innate resistance to antibiotics, and the mechanisms of virulence used by *B. cepacia* in different contexts are not well understood. This work aims to identify and describe virulence factors necessary for *B. cepacia* pathogenesis. Transposon mutagenesis in *B. cepacia* ATCC 25416, followed by a virulence assay that screened mutants using an onion infection model, was used to identify mutants of interest. A variety of phenotypes were observed, and mutant 169 was selected for follow-up studies as it generated smaller wounds as soon as 24 hours post-infection. Genetic characterizations determined that the transposon inserted in the *rfbB* gene which is the first gene in a five gene operon within chromosome 1. Analysis of cDNA generated from the operon concluded that the *rfbB* and downstream genes are not expressed in the mutant. Therefore, the inactivation of the entire operon is responsible for the observed phenotype. This gene likely encodes a virulence factor, as the disruption does not alter bacterial growth in rich media or the formation of biofilms. *rfbB* shows homology to genes encoding glucose dehydratase, which plays a role in the synthesis of the O-antigen of lipopolysaccharide (LPS). The disturbance in this outer membrane component may point towards the pathogen's inability to interact with host cells. Therefore, current work is focused on characterizing structural differences in the LPS molecules produced by the *rfbB* mutant and the wildtype strain and assessing the binding and invasion efficiency of these two strains in a plant model of infection. In doing so, this potential virulence factor can be characterized and then applied to further the understanding of the infection process.

Background

- *Burkholderia cepacia* is a primary plant pathogen of onions
- *B. cepacia* is also recognized as an opportunistic pathogen in immunocompromised humans
- Has shown the ability to colonize and infect patients with cystic fibrosis (1)
- Few virulence mechanisms utilized for pathogenesis have been characterized in recent work
- Treatment for this pathogen is difficult due to its innate resistance to antibiotics (2)

Prior Work

- The goal of this work has been to identify and characterize potential virulence-associated genes in order to understand mechanisms utilized in pathogenesis
- We performed transposon mutagenesis and screen the resulting strains through an onion infection model
- Mutants were selected for further analysis if they produced a smaller wound size following 24 and/or 48 hours
- The *B. cepacia* strain ATCC 25416 was used as its full genome is available for genome analysis

Tn 169 has a disruption in LPS biosynthesis gene *rfbB*.

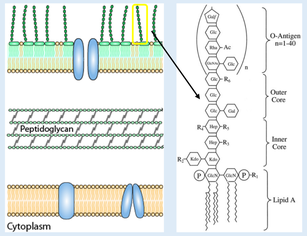


Figure 1. General gram-negative structure and LPS structure of *E. coli* O1-40 (adapted from Kadmas *et al*).

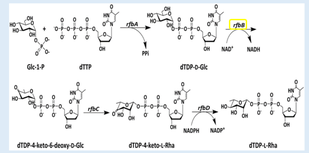


Figure 2. Predicted mechanism utilizing dTDP-glucose 4,6-dehydratase, encoded by rfbB, in the biosynthesis of dTDP-L-rhamnose (adapted from Kadmas *et al*).

Figure 3. The *rfbBDAC-DM41 operon* consisting of 5 genes which make up an operon located on chromosome 1. The transposon insertion site is located within *rfbB* which is the first gene of the operon.

- The disruption in Tn 169 resulted in decreased wounds in an onion infection model
- Tn 169 inserted into *rfbB* in the *rfbBDAC-DM41 operon* located on chromosome 1
- Tn 169 exhibits normal growth
- *rfbB* encodes for dTDP-glucose 4,6-dehydratase
- The *rfb* operon encodes for proteins necessary for lipopolysaccharide biosynthesis, specifically for dTDP-L-rhamnose synthesis
- dTDP-L-rhamnose is a structural component of the O-antigen in similar gram-negative bacteria

Tn 169 displays differences in protein and LPS composition.

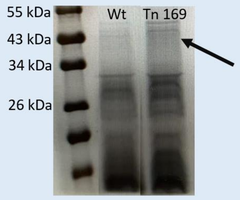


Figure 4. Protein gel of wild type and Tn 169. Visualized using coomassie blue staining.

- Whole cell lysates were used to identify differences in protein profiles between Tn 169 and wild-type *B. cepacia*
- Tn 169 has a different protein composition
- LPS was purified from both Tn 169 and wild-type strains
- LPS compositions were found to be different between the two strains

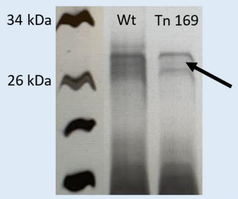


Figure 5. Protein gel of LPS extracted from wild type and Tn 169. Visualized using silver staining.

Tn 169 disrupts gene expression downstream of insertion site.

- Primers accurately amplified designated regions of the chromosome
- cDNA was generated from RNA transcripts
- All four regions are transcribed in the wild type strain
- The junction between *rfbB* and *rfbD* and the region downstream of *rfbD* are not expressed in Tn 169 mutant

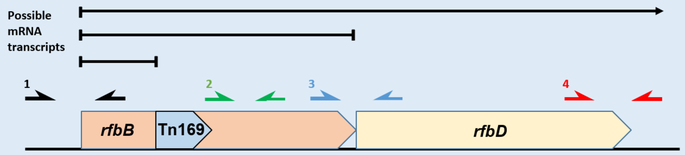


Figure 6. (Above) Possible transcript lengths resulting from potential transcription interference. (Below) Primer sets utilized in reverse transcriptase PCR to amplify upstream (black), downstream of *rfbB* (green), junction (blue), and upstream of *rdbD* (red).

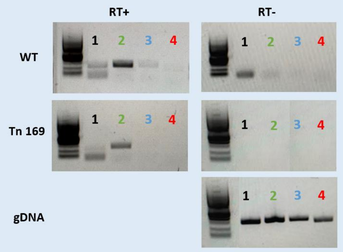


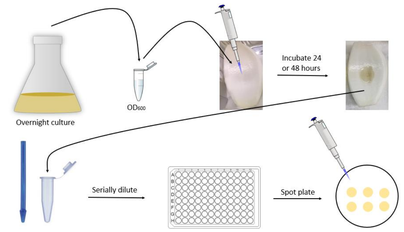
Figure 7. Gel electrophoresis for amplification of upstream region (black), downstream region of *rfbB* (green), junction (blue), and upstream of *rfbD*. Wildtype, Tn 169, and genomic DNA samples were tested either with or without reverse transcriptase (RT).

Conclusions

- Tn 169 inserted into the *rfbB* gene which caused for a smaller wound size to result in an onion infection model.
- The *rfbB* gene likely encodes for a dTDP-glucose 4,6-dehydratase necessary in LPS biosynthesis, specifically for the O-antigen subunit.
- Whole cell lysates indicate that wild-type and strain Tn 169 have varying protein compositions.
- The wild-type and Tn 169 also have differences in LPS composition, which supports the role of the *rfbB* gene product or the *rfbBDAC-DM41 operon* gene products functioning in LPS synthesis.
- The *rfbBDAC-DM41 operon* is expressed in the wild-type strain.
- Tn 169 insertion into the *rfbB* genes within the operon to not be expressed meaning their protein products are not synthesized in the mutant.

Future Directions

- Characterize function of the *rfbBDAC-DM41 operon* protein products through an onion binding-invasion assay (see below)
- Identify wild-type LPS composition then further identify structural differences in the Tn 169 mutant utilizing mass spectrometry and/or immunoassay
- Generate *rfbB* knockout and recombinant strains to test in the onion infection model



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Acknowledgements

This research was funded by the James R. Hodgson Research Fellowship and the Summer Undergraduate Research Fellowship (both at St. Norbert College).