Characterization of *thiG* Gene in *B. cepacia* as a Thiazole Synthase

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ABSTRACT

Burkholderia cepacia is a gram-negative bacterium often found in water and soil that is known to cause infection in plants and humans. *B. cepacia* was described first as the cause of sour skin rot in onion crops and later was discovered to be a human pathogen, especially in people with weakened immune systems or chronic lung diseases, such as cystic fibrosis. Limited treatments are available for this bacteria due to widespread antibiotic resistance and a lack of knowledge of the mechanisms of virulence used by *B. cepacia*. This work seeks to identify virulence factors needed for pathogenesis. Transposon mutagenesis in *B. cepacia* ATCC 25416 was used to generate mutants that were screened for defects in pathogenesis in an onion infection model of infection. A variety of phenotypes were observed and transposon mutant 370 was selected for follow-up studies. Preliminary data mutant 370 generated wounds that were distinct from wild-type wounds. This was further supported by recent data that demonstrate that the *thiG* mutant produced smaller wound sizes beginning at 48 hours post-infection, indicating that the gene product may be involved in promoting robust infection in a host. Genetic characterization of mutant 370 determined that the mutation occurred in the *thiG* gene on chromosome 1. The product of *thiG* is predicted to be a thiazole synthase, which is likely used in the metabolic processes of the bacteria and could play a role in infection. The transmembrane hydropathy plot suggests that the gene product is not a transmembrane protein and is likely found in the cytoplasm, which corresponds to its expected use in the metabolism. In support of a general role in metabolism, examination of the growth and biofilm production assays revealed that the *thiG* mutant displays stunted growth and limited biofilm production. These defects suggest that *thiG* is not involved in virulence but instead plays an important role in the basic metabolism of the bacteria. In order to clarify the function of the gene product, future research will focus on determining its involvement in metabolism using protein affinity chromatography methods. This will help to determine if the gene product interacts with another protein whose role is known with the overall goal of determining if *thiG* could be an important target for antibacterials.

BACKGROUND

Burkholderia cepacia is a bacteria predominately found in water and soil that is known to cause infection in plant and human hosts. Primarily, *B. cepacia* is known to cause sour skin rot in onion crops but has also been found to cause infection in human hosts, especially immunocompromised hosts. Limited virulence mechanisms of *B. cepacia* have been described and treatment for infections is complicated by the bacteria's inherent resistance to antibiotics. In order to develop effective treatments against *B. cepacia* infections, an understanding of the various virulence mechanisms is necessary.

The work described here aims to identify potential virulence-associated genes and characterize their work in pathogenesis. Initially, we performed transposon mutagenesis on *B. cepacia* ATCC 25416 and the resulting strains were screened using an onion infection model in order to identify genes likely involved in pathogenesis. A variety of phenotypes were observed and transposon mutant 370 was selected for follow-up studies. Genetic characterization of transposon mutant 370 determined that the mutation occurred in the *thiG* gene on Chromosome 1, which the gene product of the gene is predicted to be a thiazole synthase, which is likely used in the metabolic processes of the bacteria and may play a role in infections.

The onion infection model was repeated, and wound sizes were measured at 24-, 48-, and 72-hours post-infection. Biofilm production assays were performed using both LSLB and M63. LSLB is nutrient-rich media that contains low sodium concentrations used for growing bacterial cultures. M63 is a minimal nutrient media that contains glycerol and other carbon compounds that can be used as a carbon and energy source. M63 is often used to push bacteria through certain pathways under more limited conditions. Biofilms are a protective measure put in place by bacteria to protect themselves from antibiotics and host defense mechanisms. A growth curve was also performed using LSLB, in which the absorbance was measured every 30 minutes for 18 hours using a plate reader. Before each reading, the plate was shaken by the plate reader. Bioinformatic data was also collected on transposon mutant 370. A transmembrane hydropathy plot, a 3-D model of predicted protein structure, and a domain search were performed using the predicted amino acid sequence.

RESULTS

Which mutant(s) have a disruption in a potential virulence-associated gene?

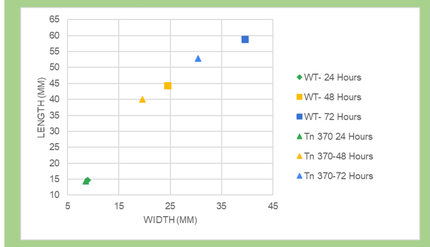


Figure 1: Wound size measurement comparison between wild type strain and Tn 370 mutant strain at 24-, 48-, 72-hours post infection.

- Mutant strain Tn 370 produced equal sized wounds as wild type strain at 24-hours post infection (hpi).
- Tn 370 produced smaller wounds than wild type strain at 48 hpi.
- Tn 370 produced smaller wounds than wild type strain at 72 hpi.
- Wound size differs more at 72 hpi than at 48 hpi.

What gene is interrupted by the inserted transposon?

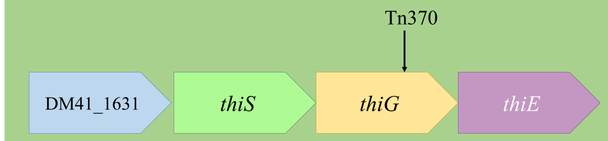


Figure 2: Schematic image of the potential operon interrupted by transposon mutant 370.

- After sequencing transposon mutant 370, we found that the transposon was inserted into the *thiG* gene on the first chromosome of *B. cepacia*.
- The predicted length of thiG is 816 base pairs long, and the estimated length of the gene product is about 272 amino acids long.
- *thiG* may be a part of an operon, as shown in the figure. The transposon interruption may play a role, not only in the function of ThiG, the but function of the operon– especially in ThiE function.

What is the *thiG* gene product predicted to look like?

- Image created by PHYRE
- 233 amino acid residues (about 86% of sequence) has been modelled with 100% confidence.
- In rainbow order from N terminus to C terminus
- Predicted to have 10 alpha helices and 12 beta pleated sheets

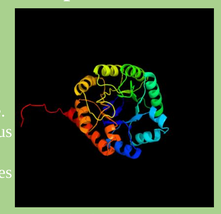


Figure 8: 3-D model of the predicted structure of the thiG gene product.

Are there predicted domains on the *thiG* gene product?

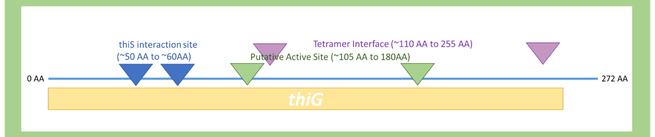
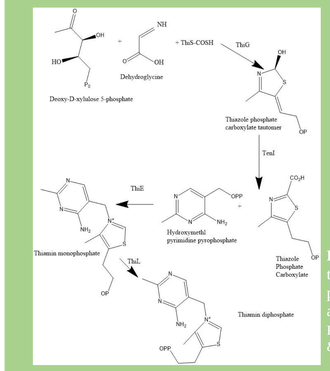


Figure 3: Schematic representation of the predicted domains present on the gene product of the *thiG* gene. Schematic draw is adapted from U.S. National Library of Medicine. (n.d.). *NCBI conserved domain search*. National Center for Biotechnology Information.

- Domain prediction was found using the amino acid sequence for the *thiG* gene product.
- The colored triangles indicate different domain regions between them. The predicted domains include an interaction site with *thiS*, an active site, and a tetramer interface domain.

What is the function of ThiG? What is affected by the insertion of a transposon into *thiG*?



- *thiG* is predicted to encode a thiazole synthase.
- In other organisms, ThiG is involved in a metabolic pathway that produces thiamin. Thiamin is also known as vitamin B-1 and has been found to be an essential cofactor and helps in energy production.

Figure 4: Reaction scheme indicating the use of *thiG* gene product in the production of thiamin monophosphate and thiamin diphosphate.
Reaction Scheme adapted from: Du, Qinglin & Wang, Honghai & Xie, Jianping. (2011).

Is there a difference between the growth of the Wild Type strain and Tn 370?

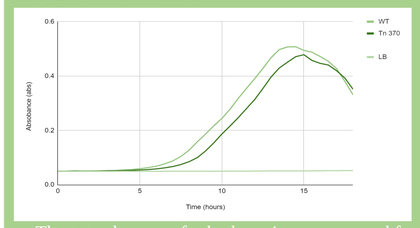


Figure 5: Absorbance over time of both wild type strain and Tn 370 strain, representing the growth of each strain over time.

- The growth curves for both strains were started from overnight liquid cultures in LSLB and kanamycin for transposon mutant 370 strain.
- Once the liquid cultures had grown to OD_{600}=1, the cultures were diluted 1:2000 and pipetted into a 96-well plate to be read at 600 nm for 18-hours every 30 minutes
- The figure indicates that the wild type strain had a higher optical density than Tn 370, indicating more growth than Tn 370.
- Both strains having similar times for peak optical densities.

Is there a difference in biofilm production between wild type strain and Tn 370? Is there a difference in production in different media?

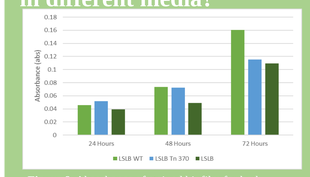


Figure 6: Absorbance of stained biofilm for both wildtype and Tn 370 at 24-,48-,72-hours in LSLB media.

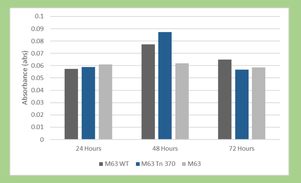


Figure 7: Absorbance of stained biofilm for both wildtype and Tn 370 at 24-,48-,72-hours in M63 media.

- Using LSLB media, Tn 370 showed slightly higher biofilm production at 24 hours than wild type. At 48 hours biofilm production is about equal between strains. At 72 hours biofilm production is greater by wild type than Tn 370.
- Using M63 media, wild type and Tn 370 are about even for production at 24 hours. Tn 370 shows slightly higher production at 24 hours. At 48 hours Tn 370 shows higher production than wild type. At 72 hours, wild type shows higher production.

CONCLUSIONS

- *B. cepacia ATCC 25416* mutant strain Tn 370 was created via random transposon mutagenesis
- Tn 370 is predicted to have inserted into the *thiG* gene, of a likely 4 gene operon.
- *thiG* gene is predicted to be a thiazole synthase, a gene product used in production of thiamin, used in metabolism.
- Tn 370 produces reduced wound sizes at 48 and 72 hpi, indicating reduced pathogenicity and further indicating that it may be involved in promoting robust infection in a host.
- The mutation affects growth in nutrient-rich media; indicating that the gene is essential.
- Both the growth curve and biofilm production assays show stunted growth and limited production, indicating that the gene is not involved in virulence, rather basic metabolism.

FUTURE DIRECTION

- Determine if the transposon insertion solely disrupted the expression of *thiG* or the expression of *thiG* and the remainder of the operon. This can be done using RT-PCR.
- Clarify the function of *thiG*, by focusing on the role of ThiG in metabolic processes.
- Determine if ThiG interacts with other proteins, whose roles are known which may help to further clarify the function of *thiG*.

SOURCES

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