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vgrG Gene as a Virulence Factor in Burkholeria cepacia

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vgrG Gene as a Virulence Factor in *Burkholderia cepacia*

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https://www.sciencephoto.com/media/486241/view/burkholderia-cepacia-bacteria

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ABSTRACT

Burkholderia cepacia is a gram-negative bacterium responsible for causing onion soft rot disease and has been identified as an infectious agent in people with cystic fibrosis. As this bacterium is naturally antibiotic resistant, it is important to understand the virulence factors that contribute to this bacterium's pathogenicity. Through the use of transposon mutagenesis and bioinformatics, the *vgrG* gene was identified as a possible virulence factor for *B. cepacia* ATCC 25416. This gene encodes the tip protein of the Type Six Secretion System (T6SS), a complex structure found in many species of bacteria. In other organisms, this syringe-like system allows a bacterium to inject proteins into neighboring cells as a mechanism of interbacterial competition or for nutrient acquisition during infection. Here, the *vgrG* gene was disrupted by transposon mutagenesis and tests were performed on both this mutant and the wild-type strain of *B. cepacia*. I have found that expression of *vgrG* is not essential for survival of *B. cepacia*, suggesting that it is used for virulence in our infection model. Similarly, the *vgrG* mutant strain created smaller lesions compared to the wild-type strain in an onion model of infection, and preliminary data suggests that this mutant cannot establish an infection without a prior wound in the host. I am currently developing genetic methods that will allow me to test whether this gene is required for bacterial interactions, such as those required for biofilm generation and interbacterial competition. Together, these data will describe how *vgrG* and T6SS contribute to the virulence of *B. cepacia*.

BACKGROUND

- The goal of this project is to identify genes in *Burkholderia cepacia* that are crucial for the infection of different hosts.
- *B. cepacia* is a gram-negative bacillus containing 3 chromosomes and is noted as a plant pathogen and opportunistic pathogen of humans. *B. cepacia* infections are especially dangerous for those with cystic fibrosis.
- *B. cepacia* is becoming increasingly antibiotic resistant, so the identification of virulence factors is important to understand in order to produce treatments can target these factors and prevent infection caused by *B. cepacia*.
- Transposon mutagenesis was used to disturb the *vgrG* gene which resides on chromosome 2 of *B. cepacia* ATCC 25146 **(Fig. 1)**.

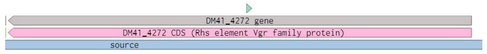

Figure 1. Site where the transposon inserted in the *vgrG* gene on chromosome 2 of *B. cepacia* ATCC 25416. Adapted from Benchling.

- The *vgrG* gene is hypothesized to encode the tip protein of the type 6 secretion system (T6SS). **(Fig. 2)**. This mutation of the *vgrG* gene likely prevents the normal function of the T6SS in *B. cepacia*.
- The T6SS can inject toxins into other cells and has been known to be used by other bacteria for interbacterial competition and to infect various hosts (Hachani *et al*, J Biol Chem, 2011).

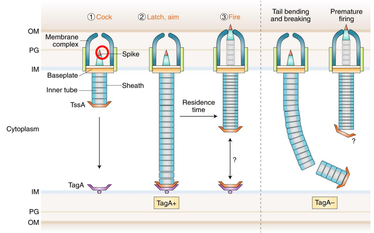

Figure 2. Diagram of the Type 6 Secretion System and its mechanism. Adapted from Francetic, nature microbiology, 2018.

- The T6SS is also known for being contact-dependent, which means that two bacterial species that are undergoing competition using the T6SS, need to be within proximity of each other **(Fig. 5 and Fig. 6)**. This proximity allows the bacteria to puncture the cell walls of their competitors, delivering deadly toxins and ultimately killing the competition.
- This process is beneficial to the bacterium as it can establish a niche during infection, and in the environment, allowing for the persistence of *B. cepacia*.

RESULTS

How well can tn415 establish infection when compared to the wild-type?

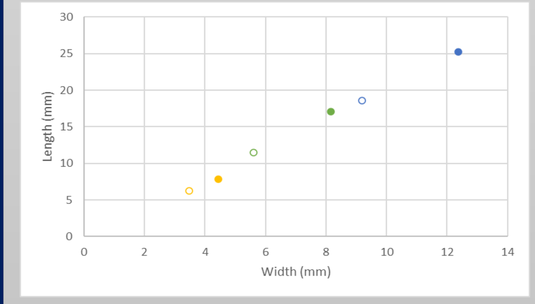

Figure 3. Onion average wound size over 72hrs. Measured in mm. Wild-type (closed circles) and tn415 (open circles). Wound size after 24hrs (yellow), after 48hrs (green), and after 72hrs (blue).

- After 24hrs, a significant difference in onion lesion size is noted. This trend continues throughout 72hrs with the difference in onion lesion size between the two strains increasing over time.
- The wild-type strain is more proficient at creating onion lesions during infection.
- The *vgrG* gene that is disturbed in the tn415 strain contributes to this strain's lack in proficiency at creating onion lesions during infection.

Is tn415 a virulence factor?

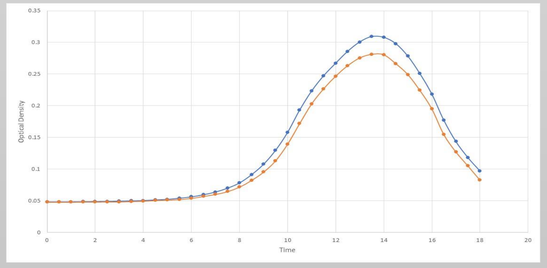

Figure 4. Growth curve analysis of wild-type strain and tn415 mutant over 18 hrs. Wild-type (in blue) and tn415 (in orange).

- These data points are not significantly different, which means that the tn415 and the wild-type strains grow similarly.
- The *vgrG* gene that is disturbed in tn415 does not have an affect on the growth of *B. cepacia*.
- The *vgrG* gene must be a virulence factor.

How do these competitions work?

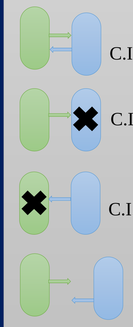

Figure 5. Diagram of possible competition outcomes. WT (green) and tn415 (blue). Black arrows indicate death while the blue and green arrows indicate the T6SS. Competitive Index (C.I.) WT/tn415.

- If both strains have a functional T6SS, the C.I. will be 1 because there will be an equal proportion of each strain after competition.
- If the tn415 strain does not have a functional T6SS due to the mutation in the *vgrG* gene, then the WT will outcompete the tn415 strain, and the C.I. will be greater than 1. (hypothesized)
- If the tn415 strain is not affected by the *vgrG* disruption, and kills WT more efficiently, then the C.I. will be less than 1.
- If both strains fail to initiate contact each other, then there will be no competition because interbacterial competition using T6SS is contact-dependent.

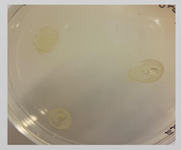

Figure 6. Bacterial competition plate.

- Bacteria is plated from a micropipette, forming a liquid drop.
- When this drop dries, most bacteria will grow along the edges of the drop.
- The edge has less space so there is more contact between the bacteria and the middle has more space so there is less contact between the bacteria.
- Since competition using the T6SS is contact-dependent, there will be more competition around the edge of the drop.

CONCLUSIONS

•The transposon insertion disrupted the *vgrG* gene on chromosome 2 of *B. cepacia.*

•The growth curve indicates that the wild-type and tn415 strains grow similarly which indicates that the tn415 mutant is not essential for the livelihood of *B. cepacia* but, instead, is a virulence factor.

•Onion infection assays indicate that the wild-type strain is far more proficient in establishing infection over the tn415 mutant with the disturbed *vgrG* gene as the wild-type produced significantly larger lesions in onion tissue over 72 hrs.

•Interbacterial competition relies on contact between strains in order to utilize the T6SS.

FUTURE DIRECTIONS

•Further onion infection data will be collected by pooling the bacteria on membrane-intact and membrane-damaged onion slices to better understand the role of the T6SS during infection with *B. cepacia.*
•More bacterial competition assays will be performed to better understand the role of the T6SS in interbacterial competition in *B. cepacia*.
•The genetic system developed in this project will be used to do complementation and gain of function experiments to better understand the role of the *vgrG* gene in the T6SS.

REFERENCES

Hachani A. *et al*. (2011). Type VI Secretion System in *Pseudomonas aeruginosa*: Secretion and Multimerization of *VgrG* Proteins. J. Biol Chem. 14: 12317-27. Doi: 10.1074/jbc.M110.193045.

ACKNOWLEDGEMENTS

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