Characterization of two putative virulence genes in *Bacillus anthracis* Sterne

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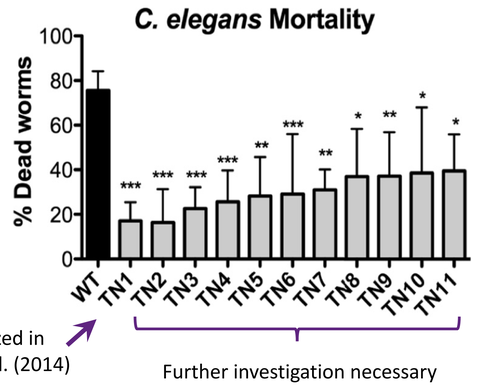
Abstract

Bacillus anthracis is a bacterial pathogen that causes the often lethal disease anthrax. This research aims to characterize the role of potential virulence genes in *Bacillus anthracis*. Virulence is a pathogen's ability to damage the host. Studying virulence allows us to understand infection mechanisms and develop novel ways to target pathogens. Previous work identified a collection of potential virulence mutants (Franks et al, 2014) each containing a genetic disruption that renders a gene non-functional. These mutants were pulled out in initial screenings but were never characterized further. We confirmed that one mutant, TN2, also exhibits decreased virulence in a *Galleria mell*onella survival assay. We know that TN2 has a disruption in a promoter region that we hypothesize controls two genes: a putative BNR repeat domain protein (2A) and a glycosyl-like 2 transferase family protein (2B). For my project, I attempted insertional mutagenesis to inactivate these genes with the goal of confirming that the genes are linked to virulence, rather than unintended mutations elsewhere in the genome. After successfully creating insertional mutant Δ2B, through the disruption of the 2B gene, I am working to further characterize the mutant to determine its role in immune evasion. Specifically, I will compare the ability of the wild-type and mutants to survive exposure to various antimicrobial defenses conserved in humans and waxworms. This research could help identify a novel bacterial virulence factor and its potential mechanisms of action thus expanding our understanding of bacterial pathogenesis.

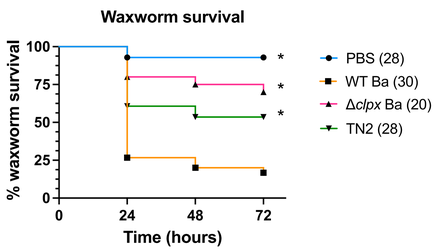
Virulence in *C. elegans*

Transposon mutants TN2-TN11 were isolated in a *C. elegans* screen. Our goal is to prioritize which mutant is important for further study and characterization.



Virulence in *G. mellonella*

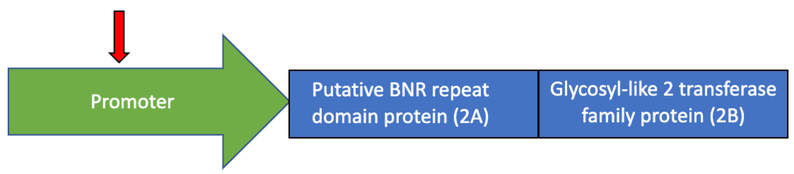
Strain (# worms)	Percent Survival at 72 hours
WT (76)	14.5
PBS (74)	71.6
Δ*clpX* (66)	51.5
TN2 (28)	53.6
TN3 (28)	35.7
TN4 (28)	25.0
TN5 (22)	9.1
TN6 (22)	4.5
TN7 (22)	0.0
TN8 (34)	26.5
TN9 (12)	25.0
TN10 (34)	29.4
TN11 (12)	0.0
TN12 (12)	0.0
TN13 (12)	0.0



* indicates p<0.01 from survival with WT Ba using the log-rank test

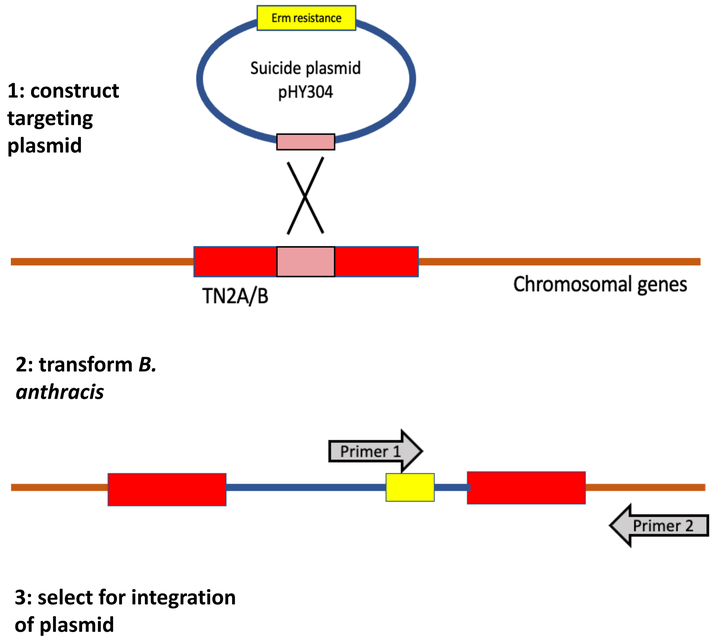
TN2 is attenuated in the invertebrate model *G. mellonella* in addition to *C. elegans.*

Transposon Insertion Site



TN2 insertion is located in a promoter region controlling two genes, putative BNR repeat domain protein and glycosyl-like 2 transferase family protein.

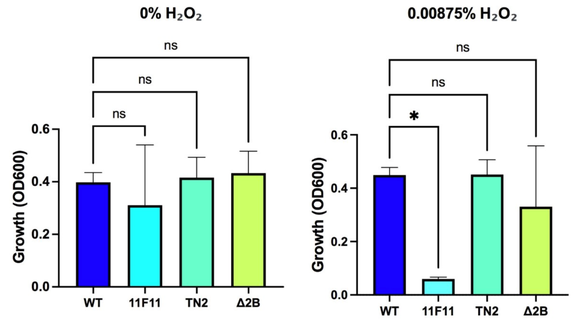
Construction of Insertional Mutant



Confirmation of Insertional Mutant

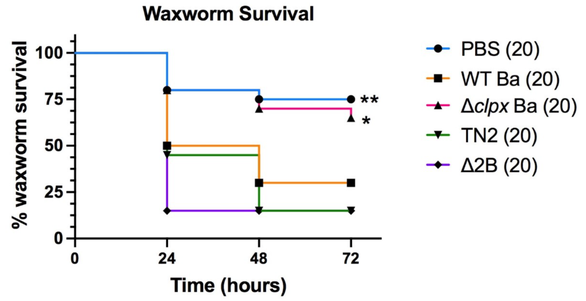
An insertional mutant interrupting 2A was unable to be constructed. However, an insertional mutant containing an interruption in the the glycosyl-like 2 transferase family protein (2B) was successfully constructed. Integration was confirmed via PCR amplification.

Hydrogen Peroxide MIC Assay



There is no statistically significant difference in growth of TN2 or Δ2B when compared to WT in our hydrogen peroxide MIC assay, suggesting that glycosyl-like 2 transferase family protein does not seem to neutralize reactive oxygen species.

Survival Assay with Insertional Mutant



* indicates p<0.01 from survival with WT Ba using the log-rank test

Conclusions and Future Directions

Initially, TN2 seemed to be attenuated in two invertebrate infection models. Later *G. mellonella* survival assays showed this phenotype did not remain. Thus, further investigation is necessary to confirm the accuracy of the *G. mellonella* survival assay and to determine whether the initial attenuated phenotype observed in TN2 is legitimate. We successfully interrupted the glycosyl-like 2 transferase family protein gene, which is the second of two genes in the operon. In the future, we will use insertional mutagenesis to inactivate the first gene as well as both genes together. We also hope to utilize complementation to attempt to restore the wild-type phenotype in TN2. Our next step will be to use these insertional mutants to perform additional MIC assays using antimicrobial peptides.

Acknowledgments

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References

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