Characterizing potential virulence genes in *Bacillus anthracis* Sterne using invertebrate models of infection

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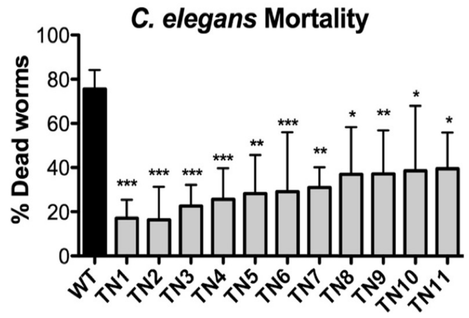
Abstract

The gram-positive bacterium, *Bacillus anthracis,* is responsible for the deadly disease Anthrax. *B. anthracis* is dangerous due to virulence factors, or defenses the bacteria uses to infect a host. We hope to better understand how this bacterium interacts with its hosts by studying the genes necessary for virulence. Bacterial mutants, which have a change in their genetic sequence, sometimes show reduced ability to cause disease in a host. Studying these mutants helps us understand the bacteria's infection method. Previously our lab created a library of mutants using a technique called transposon mutagenesis and then screened these transposon mutants for phenotypes linked to decreased virulence. This resulted in the identification of 11 transposon mutants that were less effective at causing disease in the nematode *Caenorhabditis elegans* (Franks et al.). While all 11 mutants could be interesting for further characterization, it is necessary to prioritize them as this is still too many to study. In this project, we tested these mutants using a second infection model, the caterpillar *Galleria mellonella*. *G. mellonella* is an ideal model due to its optimal size for injection, conserved innate immune defenses, and previous success as an infection model for *B. anthracis* (Malmquist et al.). We found that only one of these 11 mutants, TN2, had reduced virulence in both *C. elegans* and *G. mellonella*. Future research will focus on confirming the genetic change in this mutant and determining the mechanism by which it contributes to infection. This could lead to new antibiotic targets in the future.

Background



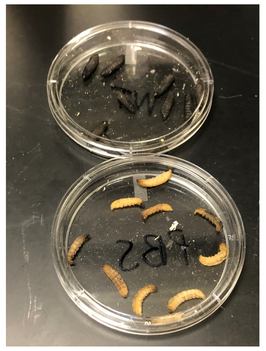
Characterized in Franks et al. (2014)

Mutant	Disrupted gene[a]	Gene symbol
TN1	Putative tellurite resistance gene	BAS0389
TN2	BNR repeat domain protein	BAS2814
TN3	Conserved hypothetical protein	BAS0642
TN4	Protease production regulatory protein	BAS0976
TN5	Hypothetical protein	BAS0432
TN6	Spore germination protein	BAS3369
TN7	Conserved hypothetical protein	BAS0645
TN8	Sensor histidine kinase	BAS0869
TN9	PTS, IIB component	BAS2290
TN10	Acetyl-CoA hydrolase	BAS1726
TN11	Oxidoreductase	BAS0712

Further investigation necessary to determine function

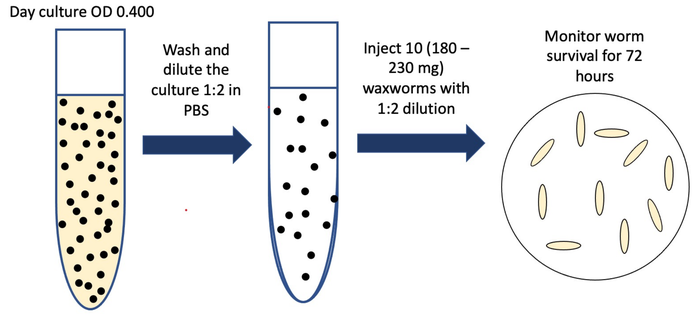
Transposon mutants TN2-TN11 were isolated in a *C. elegans* screen. Our goal is to prioritize which mutant is important for further study and characterization.

Galleria mellonella Model

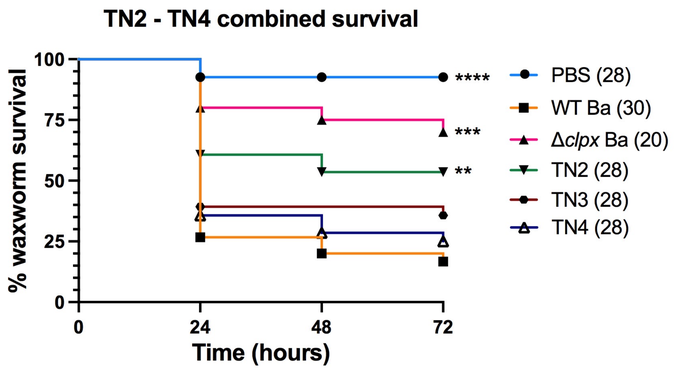


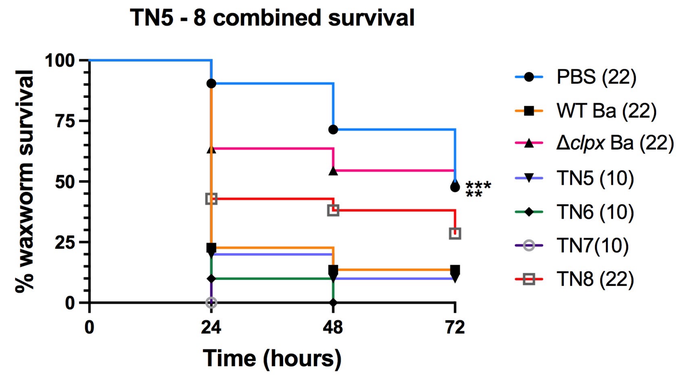
G. mellonella characteristics:

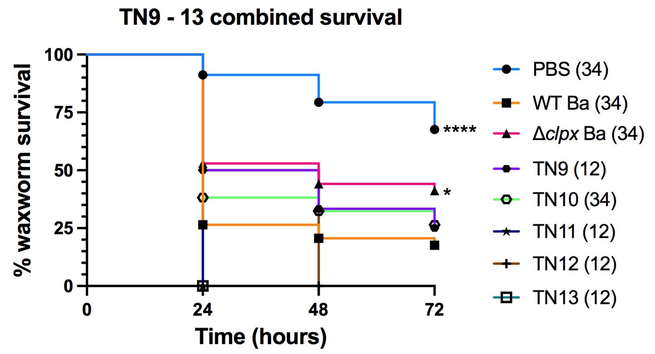
- Conserved innate immune defenses
- Can be incubated at 37 ºC
- Injectable size
- Inexpensive
- Easy to determine survival



Survival Assay Screens



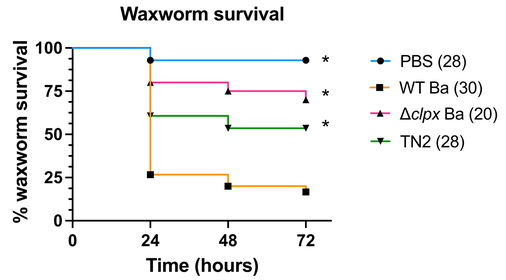




*Indicates statistically different survival than WT, p<0.01 using log-rank test for each graph.

TN2 is Attenuated in Waxworm Model

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



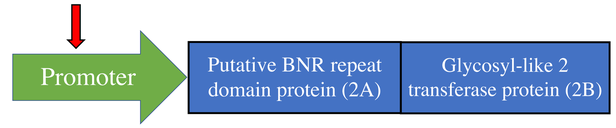
* indicates p<0.01 from survival with WT Ba using the log-rank test

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

Conclusions & Future Directions

TN2 is attenuated in both the invertebrate model *C. elegans* and the wax worm *G. mellonella*. Future directions will be to investigate the genes disrupted.

Site of transposon insertion in TN2



References

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Acknowledgements: Funding for this project was provided by TCU SERC grant to Bella Kouretas.

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