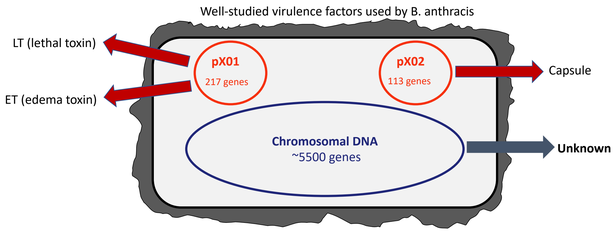
Screening for Novel Virulence Factors Using H_2O_2 in *Bacillus anthracis* Sterne

Abi Plylar, Luke Hamilton, and Shauna M. McGillivray
Department of Biology, Texas Christian University, Fort Worth, TX

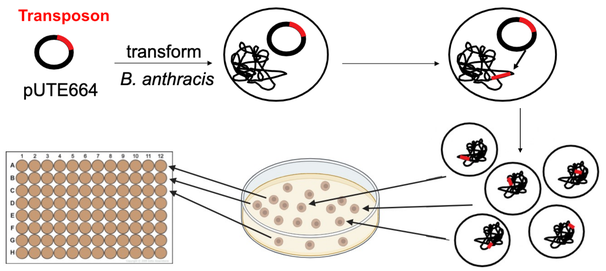
I. Background

Bacillus anthracis is a spore-forming bacterial pathogen and the causative agent of the deadly disease, anthrax.

Why is the *B. anthracis* infection so lethal?

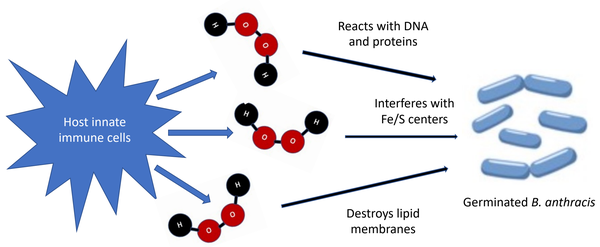


Objective: Identify novel chromosomal virulence genes by screening transposon mutants against H_2O_2 and validating these screens



Why challenge mutants against H_2O_2?

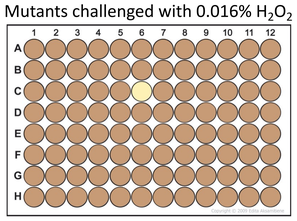
H_2O_2 is released by macrophages and neutrophils in the host's first line of defense against invading microbes because it reacts with important structures needed for pathogen survival.



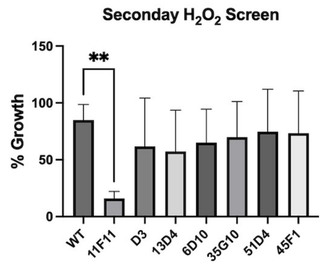
References: McGillivray, S.M., et al., *ClpX Contributes to Innate Defense Peptide Resistance and Virulence Phenotypes of Bacillus anthracis</i>.* Journal of Innate Immunity, 2009. **1**(5): p. 494-506.

II. *In-vitro* H_2O_2 Screen

Mutants challenged with 0.016% H_2O_2



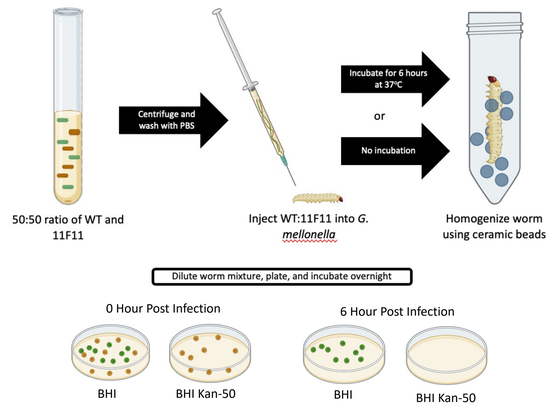
OD analyzed to observe mutants with decreased survivability



Conclusions:

- *In-vitro* screen validated through identification of 11F11, but this mutant has a disruption in the catalase gene, which is not a novel virulence factor
- Screen reveals many false positives

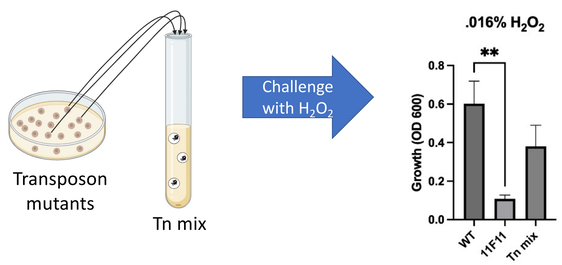
III. Validate *In-vivo G. mellonella* Screen



Question: Are mutants exhibiting increased susceptibility to there is a transposon present in the genome, or because the location of the transposon is in a gene related to H_2O_2 virulence?

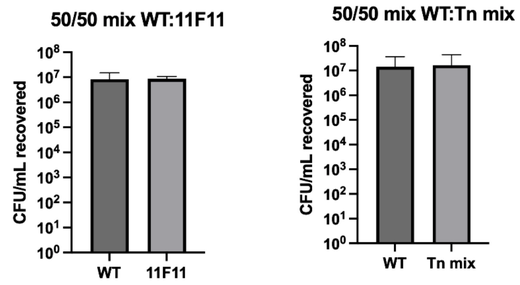
IV. Developing a Control Transposon Mix

Hypothesis: The Tn mix should exhibit a WT-like phenotype since most of the mutants in the mix will not have a mutation affecting H_2O_2 response

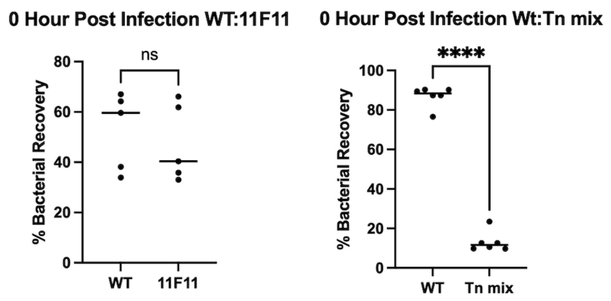


V. Confirming Tn mix Control *In vivo*

Amount of bacteria before worm injection



Amount of bacteria after worm injection



V. Conclusions and Future Directions

- *In vitro* the Tn mix exhibits growth similar to WT
  - The presence of a transposon does not seem to be responsible for loss of H_2O_2 resistance
- Preliminary data shows that bead beating may kill the Tn mix bacteria,
  - This would account for the loss of bacterial recovery after the 0 hour incubation
- Repeat *in vivo* competition assay to confirm bead beat results
- Complete *in vivo* competition assay to confirm *in vitro* Tn mix results

Acknowledgements: Funding for this project was provided by TCU SERC grants to Abi Plylar

reSEaRCh
Science and Engineering Research Center