Identifying Novel Genes with Increased Susceptibility to Reactive Oxygen Species in *Bacillus anthracis* Sterne

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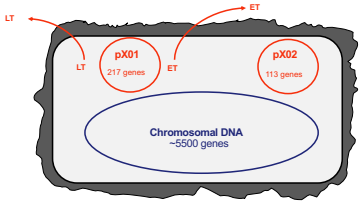
Abstract

Bacillus anthracis is a gram-positive bacterial pathogen that causes the deadly infectious disease anthrax. *Bacillus anthracis* contains two plasmids, pX01, and pX02. These plasmids were found to be necessary for the virulence of *B. anthracis*. However, *Bacillus anthracis* contains over 5,000 chromosomal genes and we believe that there are additional virulence genes that have yet to be discovered. Our lab constructed a transposon mutant library with random disruptions in the *B. anthracis* Sterne genome to screen for novel virulence factors. This library has been successfully used to identify the chromosomal genes *clpX* and *yceGH* and show their importance for *B. anthracis* virulence. To find additional novel virulence genes, we used the same transposon library and screened around 1,000 mutants using hydrogen peroxide, a reactive oxygen species (ROS). ROS are involved in the immune defense and the mutants that are attenuated in its presence may have a disrupted gene that contributes to the pathogenicity of *B. anthracis*. We obtained two mutants that were repeatedly susceptible to hydrogen peroxide *in vitro*. To determine the virulence of these mutants in an animal model, we will be performing an *in vivo* assay using the waxworm, *Galleria mellonella*. Mutants that have reduced virulence in *G. mellonella* will then be further tested to determine the location of the transposon in the genome to find out which genes are disrupted. The findings of this research could be used as potential therapeutic drug targets and could offer insight into the mechanisms that *B. anthracis* uses for its pathogenesis.

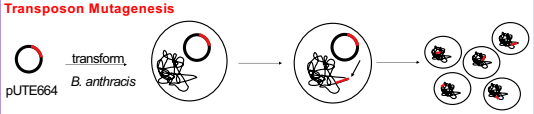
Bacillus anthracis genome



- pX01 produces the lethal and edema toxin which interferes with immune cell signaling
- pX02 produces the capsule and inhibits phagocytosis

Question: What role do chromosomal genes play in pathogenesis?

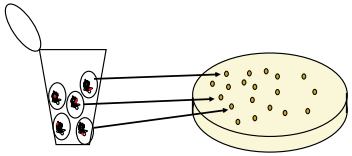
Transposon Mutagenesis



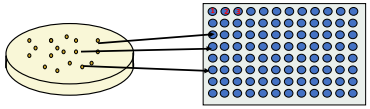
Objective: To identify novel chromosomal genes with increased susceptibility to hydrogen peroxide using a *B. anthracis* transposon mutant library

Procedure

Day One: Plate Transposon mutants on Kan50 plates



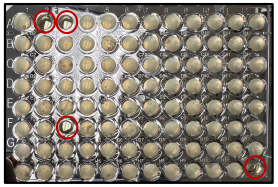
Day Two: Pick colonies into 96-well plate with BHI



Well 1: WT
Well 2: 4D5*
Well 3: 11F11*
Wells 4-95: TN mutants
Well 96: Blank
** positive controls*

Day Three: Challenge with H_2O_2

Day Four: Check growth after overnight incubation



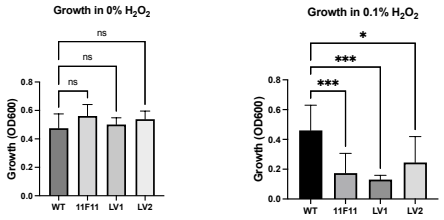
Representative image depicting growth of the transposon mutants in a 96-well plate in the presence of H_2O_2. Red circles represents no growth. Wells 2 and 3 are the positive controls known to have decreased resistance to H_2O_2.

Results: Each plate of colonies were tested at least twice and mutants that showed reduced growth were re-tested multiple times.

From the 1,000 mutants screened, two were consistently attenuated and were named LV1 and LV2.

LV1 and LV2 mutants are attenuated

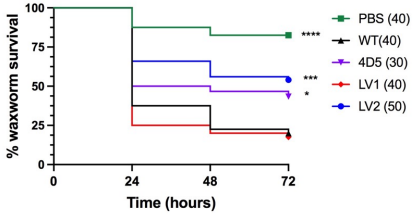
MIC assay performed on LV1 and LV2



* Indicates one-way ANOVA followed by the Dunnett's post-hoc test

Role in Virulence

Waxworm Survival



* indicates p<0.05, *** indicates p<0.001, **** indicates p<0.0001 from WT Ba survival using the log-rank test

Conclusions and Future Directions

- We optimized screening conditions for an *in vitro* H_2O_2 susceptibility assay and screened 1000 random transposon mutants.
- Two mutants, LV1 and LV2, were identified with increased susceptibility to H_2O_2. This indicates a disruption in a gene that is important for resistance to H_2O_2.
- LV2 shows attenuation in the *in vivo G. mellonella* assay. This indicates a disruption in a gene that is necessary for virulence.
- Future directions include finding the site of the transposon insertion in LV2.

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