Development and use of a *G. mellonella* infection model to discover novel virulence mutants in *B. anthracis*

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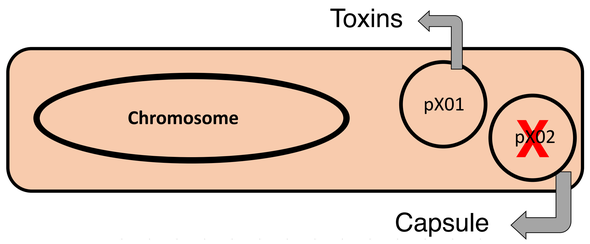
Introduction

- Understanding bacterial disease mechanisms is important because it can uncover new potential antibiotic targets[1,2].
- *In vivo* bacterial virulence is currently assessed in the mouse (*Mus musculus*) model. When working with this vertebrate organism, there are extensive infrastructure, cost, time, and ethical requirements.
- As an invertebrate, *G. mellonella* does not have the same requirements as a mammalian model.
- Previous studies have investigated the potential use of the larvae of the greater wax moth (*G. mellonella*) as an *in vivo* model for both fungal and bacterial pathogens with relative success[3,4].

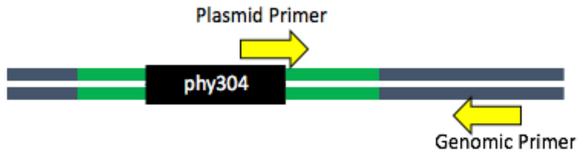


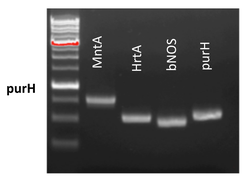
- *Bacillus anthracis* is a bacterial pathogen that has yet to be studied in this invertebrate model.
- In order to develop a model organism that is more laboratory amenable, this study had two objectives:
  1) To validate *G. mellonella* as an appropriate model for *B. anthracis* mutants
  2) To use this model to identify novel virulence genes

Methods

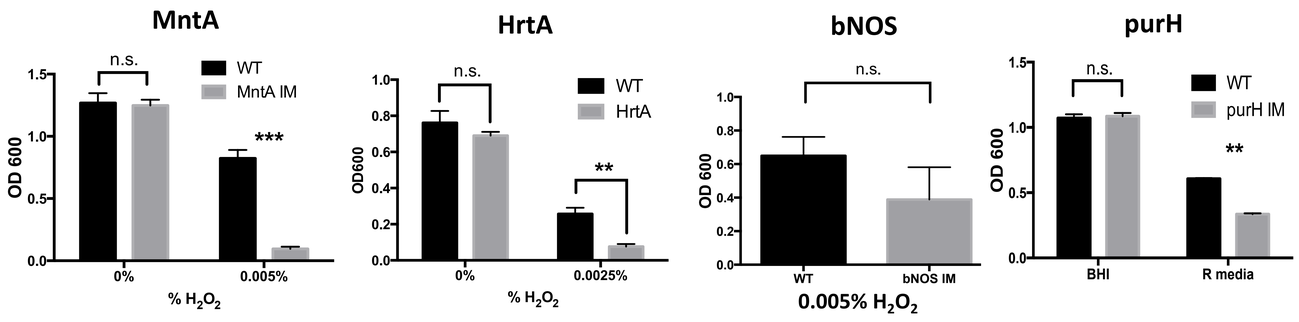
Known *B. anthracis* virulence mutants were used to validate *G. mellonella* as an *in vivo* infection model.

- A total of seven mutants were assessed in *G. mellonella.*
  - Three of these mutants were previously generated and studied in our lab (*ΔpX01, ΔclpX,* and *ΔyceGH).*
  - Four additional mutants were constructed through insertional mutagenesis (*MntA IM*, *HrtA IM*, *bNOS IM*, and *purH IM*).





- Constructed mutants were then evaluated for a comparable phenotype to what was described in the literature.

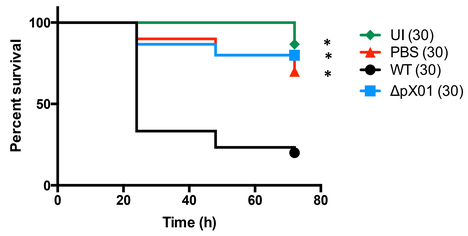


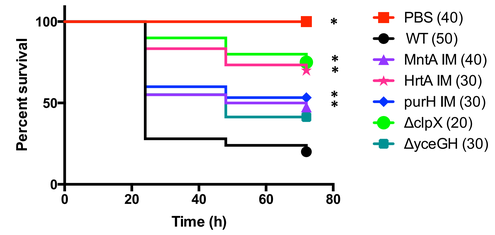
- Early log phase mutants were injected at 1:2 dilution into *G. mellonella.* Survival of *G. mellonella* was observed over 72 hours.

References: *(1)* Palumbi, S. R. (2001). Humans as the world's greatest evolutionary force. *Science* (New York, N.Y.), *293*(5536), 1786-1790. *(2)* Clastworthy, A.E., Pierson, E., &Hung, D.T. (2007). Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology*, *3*(9), 541-548. *(3)* Mylonakis, E., Moreno, R., Khoury, J. B., Idnurm, A., Heitman, J., Calderwood, S. B., ... Diener, A. (2005). Galleria mellonella as a Model System To Study Cryptococcus neoformans Pathogenesis. *Infection and Immunity,73*(7), 3842-3850. *(4)* Peleg, A. Y., Sebastian, S., Monga, D., Eliopoulos, G. M., Moellering Jr., R.C., Mylonakis, E. (2009). *Galleria mellonella* as a Model System To Study *Acinetobacter baumannii* Pathogenesis and Therapeutics. *53*(6), 2605-2609

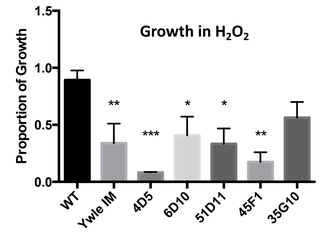
Results

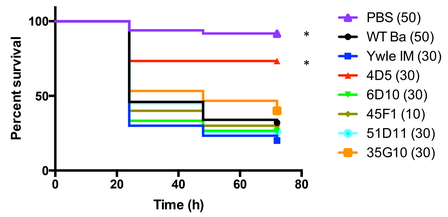
Objective 1: Validate *G. mellonella* as an *in vivo* model by assessing known virulence mutants.

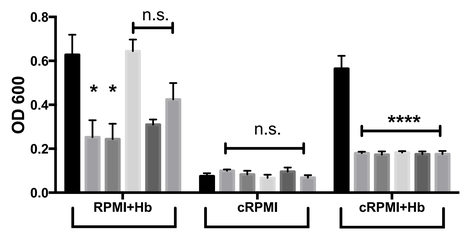


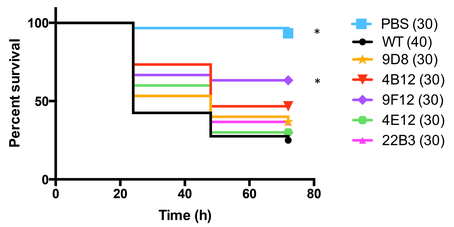


Objective 2: Screen transposon library and assess potential virulence mutants *in vivo.*

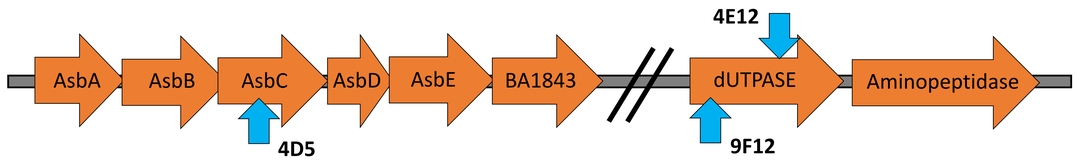








Transposon insertion site for 4D5, 9F12, and 4E12 mutants



Conclusions and Future Directions

- ***G. mellonella* is capable of discerning attenuated virulence phenotypes and therefore, could be a reasonable alternative for *in vivo* infection studies.**
- Further studies should be performed utilizing mutants whose mode of action operated on different pathways (ex. antimicrobial peptides, phagocytic cells).
- A large scale *in vitro* screen of the remainder of transposon library will be performed. Mutants with *in vitro* phenotype will be assessed in *G. mellonella* for *in vivo* virulence.

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