The characterization of the potential iron-acquisition gene *dUTPase* in *B. anthracis*

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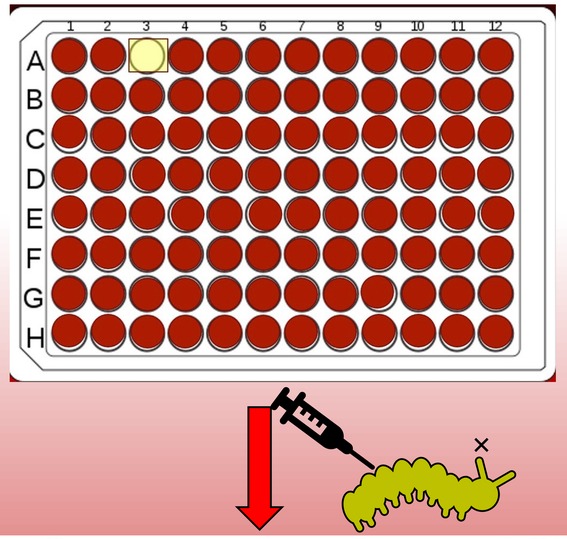
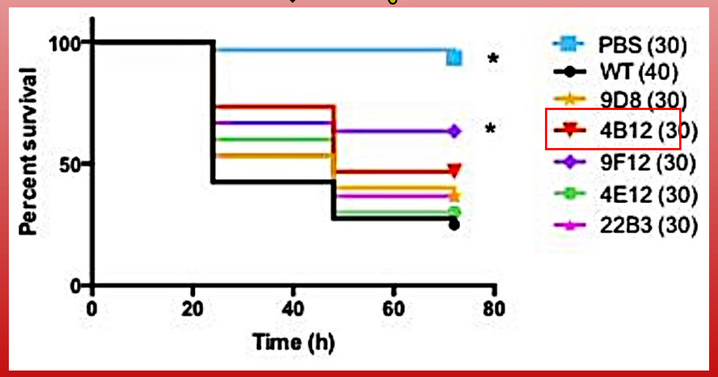
Background

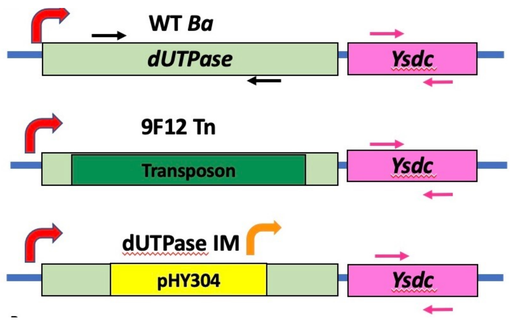
B. anthracis is recognized as the causative agent of the disease anthrax and has the potential to be used as a biological weapon. Thus, there is must interest in studying its virulence. The most characterized virulence factors are the pXO1 and pXO2 plasmids. However, there has been a shift in interest into the characterization of novel virulence factors encoded in the chromosome. We chose to focus our research on chromosomal genes associated with the acquisition of the essential micronutrient iron. Bacteria use iron for cellular functions such as growth, DNA replication, metabolism, and energy generation; however, iron is only found in the external environment. To survive, *B. anthracis* must obtain iron from its host's hemoglobin. Several genes have shown to be important for iron acquisition from hemoglobin. However, the deletion of these genes resulted in have no attenuated virulence phenotype *in vivo*. This has sparked interest in the discovering of other chromosomal genes associated with iron acquisition from hemoglobin that may also contribute virulence. Therefore, the goal of this study is to use the 9F12 Tn mutant and an independently created insertional mutant to confirm whether the *dUTPase* gene is necessary for *B. anthracis'* ability to acquire iron from hemoglobin and whether it is important for virulence in *B. anthracis*

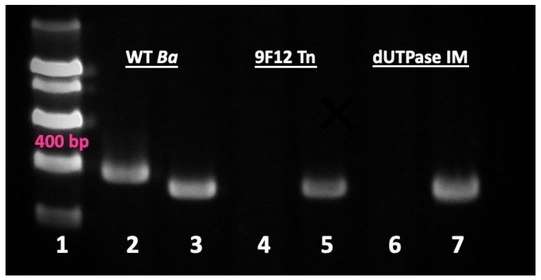
Finding of 9F12 Tn



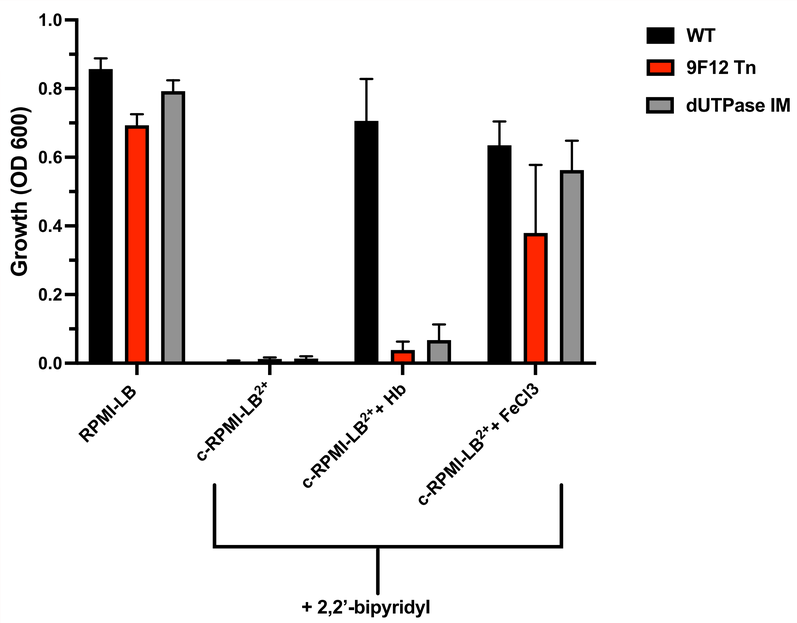


Confirmation of disrupted *dUTPase* gene



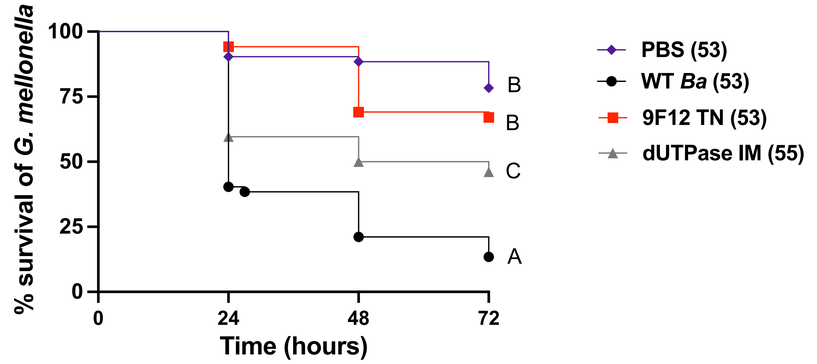


The *dUTPase* gene is necessary for growth with hemoglobin as an iron source



Wild-type *B. anthracis* (WT *Ba*), 9F12 Tn, and dUTPase IM were grown in RPMI-LB, or c-RPMI-LB supplemented with iron-free divalent cations (c-RPMI-LB^{2+}). The addition of 2,2'-bipyridyl, hemoglobin (+Hb) or iron (+$FeCl_3$) to the media is indicated Data is presented as mean +/- SEM from 4 independent experiments.

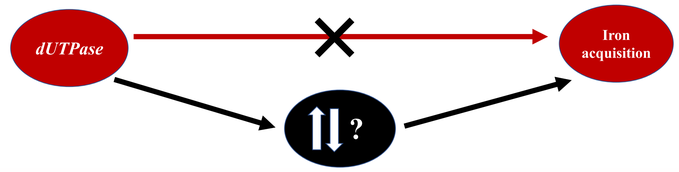
The *dUTPase* gene is necessary for *B. anthracis virulence*.



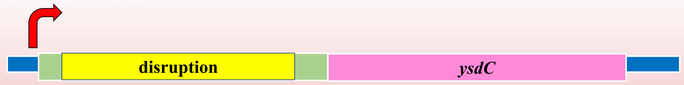
Percent survival of *G. mellonella* injected with PBS, wild-type *B. anthracis* (WT *Ba*), 9F12 Tn, dUTPase IM at 24, 48, and 72 hours. Each infection was repeated 5 independent experiments with the total number of worms for each condition in parentheses. Same letters indicate $p > 0.01$, different letters indicate $p < 0.01$ using the log-rank test.

Future Directions

dUTPase?

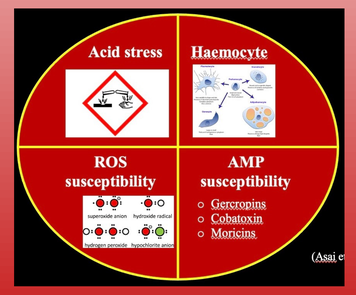


Does *ysdC* have a role?



- RT PCR to observe how much *ysdC* is being transcribed
- Create complementation mutant to confirm phenotype seen in wild-type
  - p*dUTPase*
  - p*dUTPase*/*aminopeptidase*
  - p*ysdC*

Galleria?



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