The Identification of Novel Genes Related to Iron Acquisition in *Bacillus Anthracis* Sterne

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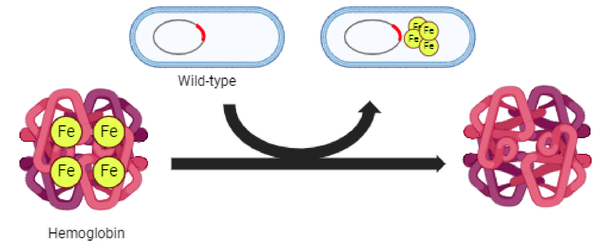
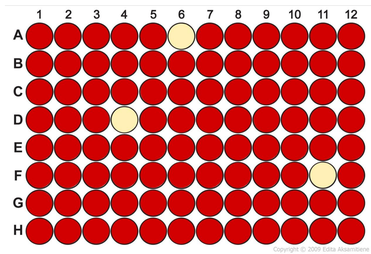
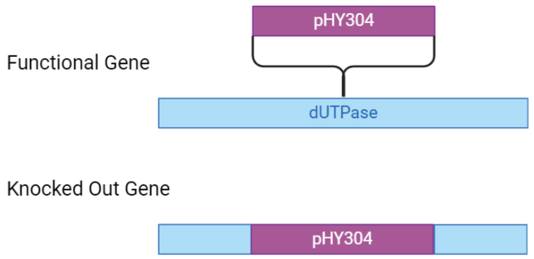
BACKGROUND

Bacillus anthracis, the causative agent of anthrax, is a spore-forming, gram-positive bacterium. Its virulence mechanisms are of interest due to its potential use as a biological weapon and high lethality. For *B. anthracis* to survive and reproduce in a host, it must evade the host's immune response and acquire nutrients. One important nutrient *B. anthracis* must acquire is iron. Iron is a limiting nutrient in the host because it is usually found sequestered to hemoglobin or bound to host proteins such as transferrin. To acquire iron, pathogens must strip it from the host proteins. To find genes important for iron acquisition from hemoglobin, we screened genetic mutants created through transposon mutagenesis. Media was chelated to remove all divalent cations, including iron, and then hemoglobin was added as the sole iron source. The mutants that were unable to grow were chosen to be tested in a larger volume hemoglobin assay. We confirmed the phenotype of several mutants using this larger volume assay and we are working to confirm the site of transposon disruption via PCR. The mutants identified include a mutation in a dUTPase gene and an L-aspartate oxidase gene, neither of which has been previously linked to iron acquisition from hemoglobin.



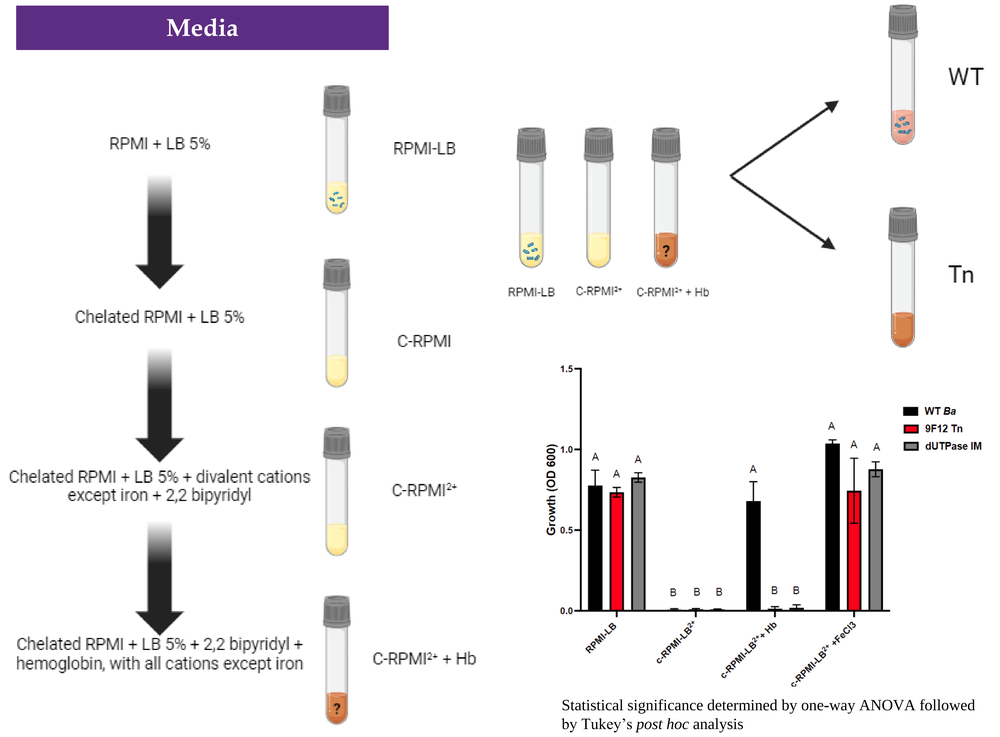


Transposon Mutants	
9D8	22B3
4B12	22B5
4E12	3A8
9F12	1B11



METHODS

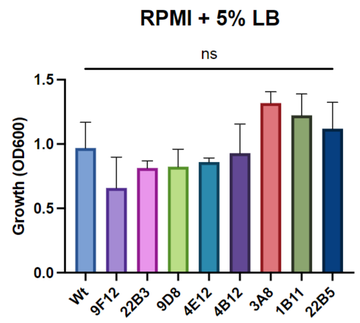
Media



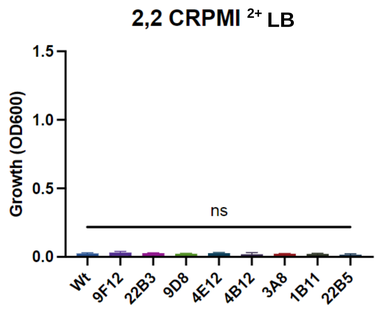
Statistical significance determined by one-way ANOVA followed by Tukey's *post hoc* analysis

RESULTS

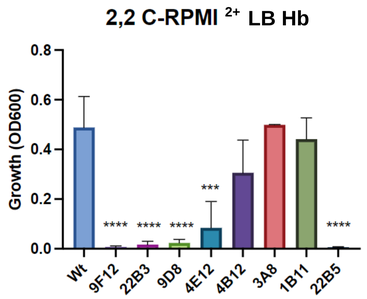
Hemoglobin Assay



Statistical significance from wild-type growth determined by one-way ANOVA followed by Dunnett's *post hoc* analysis

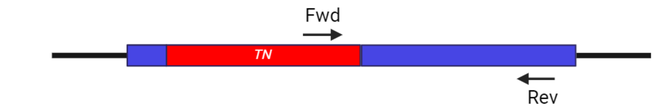


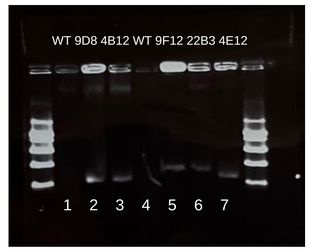
Statistical significance from wild-type growth determined by one-way ANOVA followed by Dunnett's *post hoc* analysis \

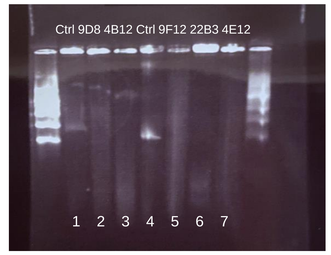


Statistical significance from wild-type growth determined by one-way ANOVA followed by Dunnett's *post hoc* analysis

PCR CONFIRMATION

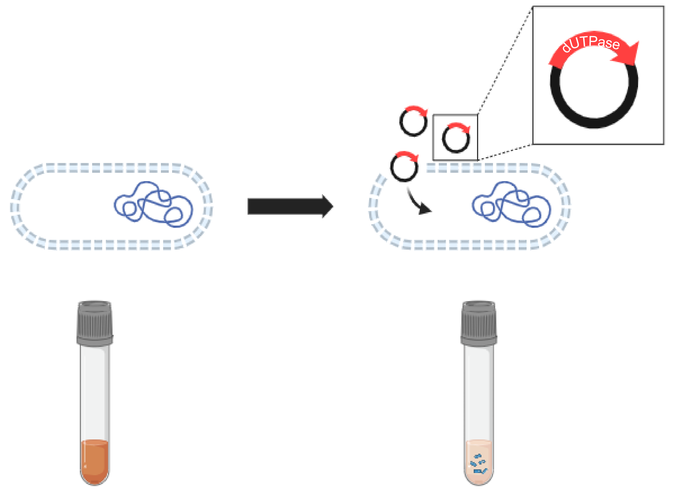






Tn Mutant	Gene Disrupted	Phenotype in Hb	Tn Confirmed
9F12, 22B3	dUTPase	No growth	Yes
4E12	dUTPase, further downstream	No growth	Yes
9D8, 4B12	L-aspartase oxidase	9D8 - No growth 4B12 - Growth	Yes

FUTURE DIRECTIONS



- Continue to test mutants for the phenotype of those that lose the ability to acquire iron
- Complement and/or make insertional mutations of all the independent mutants to confirm the phenotype

ACKNOWLEDGEMENTS

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