Identifying Novel Mutants with Increased Susceptibility to H_2O_2 and Reduced Virulence in *Bacillus anthracis* Sterne

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Bacillus anthracis is a gram-positive bacterial pathogen that causes the deadly infectious disease anthrax. *B. anthracis* contains over 5,000 chromosomal genes, and we believe there are unidentified chromosomal genes important for virulence. Our lab constructed a transposon mutant library with random disruptions in the *B. anthracis* Sterne genome to screen for novel virulence factors, and we have previously identified two virulence genes*, clpX* and *yceGH,* using this library. In this screen, we used H_2O_2, a reactive oxygen species involved in innate immune defense, and screened around 1000 mutants. We obtained three mutants that were susceptible to hydrogen peroxide *in vitro*: 11F11, LV1, and LV2. To determine whether they also had phenotypes *in vivo*, we infected *Galleria mellonella* to study their virulence in an invertebrate animal infection model. LV2 showed reduced virulence in the *in vivo* survival assay, and all three mutants showed reduced virulence in the *in vivo* competition assay. I have determined the site of the transposon insertion in 11F11 and LV1, and the transposon has inserted in the genes for catalase and a collagenase-like protein, respectively. I am currently creating an independent insertional mutation in LV1 to confirm that the observed phenotypes are linked to the disruption of the collagenase-like protein. Future directions include creating a complementation plasmid for LV1 and determining the insertion site of LV2. The findings of this research could be used as potential therapeutic drug targets and will offer insight into the mechanisms that *B. anthracis* uses for its pathogenesis.