Role of *sigM* and *glpF* on antimicrobial resistance and virulence in *Bacillus anthracis*

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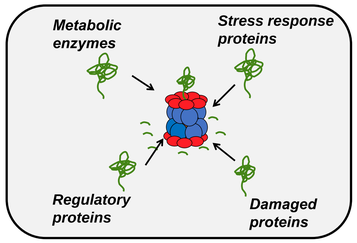
Introduction

Bacillus anthracis is a gram-positive bacterium that causes the deadly anthrax disease. ClpX is a subunit of ClpXP protease that is known to be essential in virulence as well as providing resistance to cell-envelope targeting antibiotics such as penicillin, daptomycin, and the antimicrobial peptide LL-37. While *clpX* is critical for virulence in *B. anthracis*, it is unlikely to be directly mediating the effect. Hence, our lab investigated the genes that are differentially expressed in the Δ*clpX* mutant compared to the wild type *B. anthracis* through microarray analysis. We found 119 genes that were highly differentially expressed in the Δ*clpX* mutant. In this study, we focused on two genes *sigM* and *glpF*, which are downregulated in the Δ*clpX* mutant, because *sigM* and *glpF* confer resistance to cell-wall targeting antibiotics in the closely related gram-positive bacterial species, *Bacillus subtilis* and *Staphylococcus aureus* respectively. We wanted to determine whether loss of *sigM* and *glpF* will lead to similar phenotypes as loss of *clpX* in *B. anthracis* Sterne

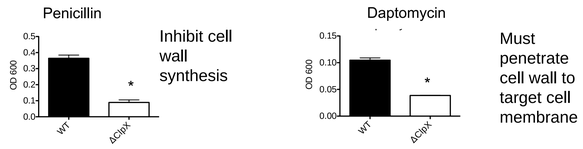
ClpXP protease



ClpX: regulatory ATPase
Recognizes and unfolds proteins
ClpP: proteolytic core
Degrades proteins

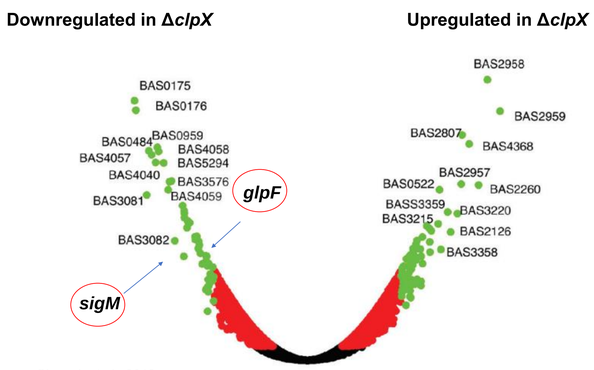
Loss of Clp protease can have pleiotropic effects on bacterial cell

Antimicrobial susceptibility of Δ*clpX*



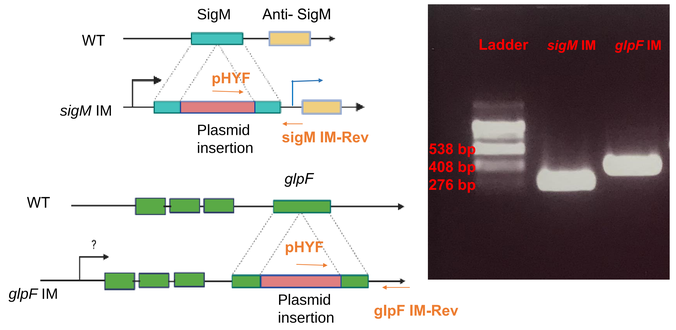
Modified from *Zou et al., 2021*

Differentially expressed genes in Δ*clpX*

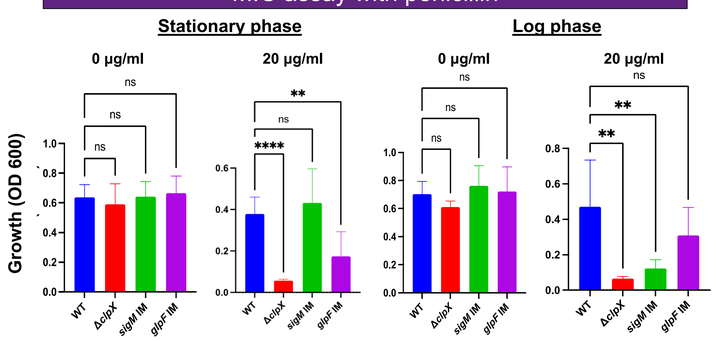


Claunch et al., 2018

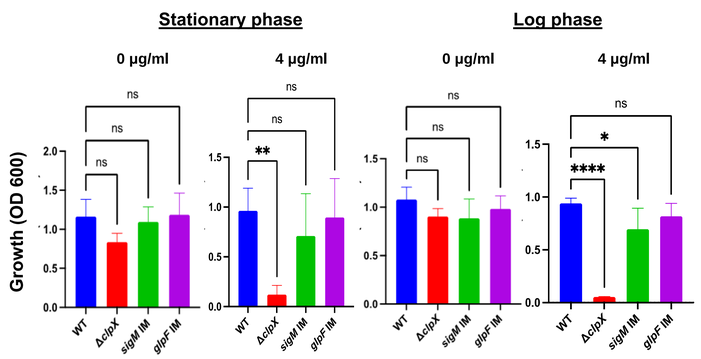
Construction of insertional mutants



MIC assay with penicillin



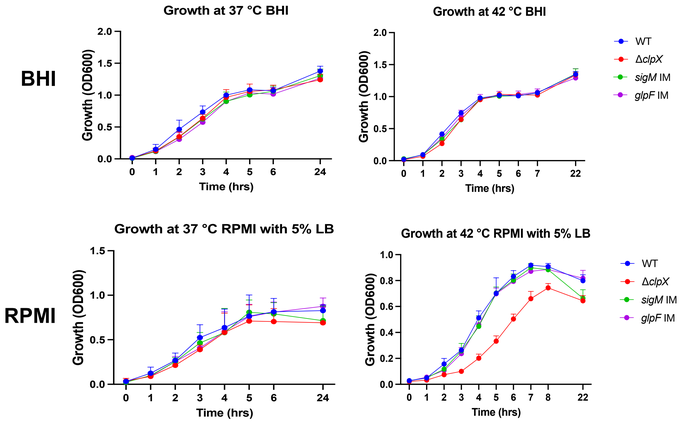
MIC Assay with daptomycin



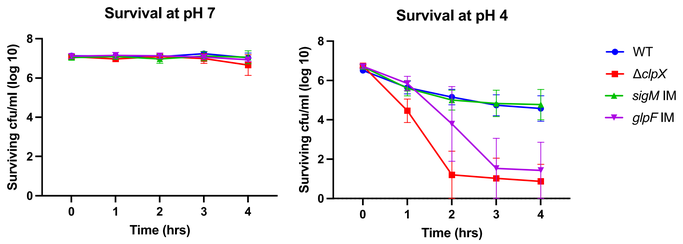
References

McGillivray, S. M., Tran, D. N., Ramadoss, N. S., Alumasa, J. N., Okumura, C. Y., Sakoulas, G., Vaughn, M. M., Zhang, D. X., Keiler, K. C., & Nizet, V. (2012). Pharmacological Inhibition of the ClpXP Protease Increases Bacterial Susceptibility to Host Cathelicidin Antimicrobial Peptides and Cell Envelope-Active Antibiotics. *Antimicrobial Agents and Chemotherapy*, *56*(4), 1854–1861. https://doi.org/10.1128/AAC.05131-11

Growth curve



Acid stress



Conclusions

- *sigM* IM is more susceptible to daptomycin and penicillin, although in a growth phase dependent manner.
- *glpF* is critical for penicillin resistance in stationary phase.
- *sigM* and *glpF* are not critical in providing tolerance to heat stress.
- Loss of *glpF* increases susceptibility to acid stress.

Future directions

- Future studies will examine the susceptibility of *sigM* IM and *glpF* IM mutants to antibiotics like LL-37 and vancomycin.
- Complementation of these mutants will serve to further support the importance of these genes for the roles we examined.
- This research will aid in understanding the mechanism of antibiotic resistance and virulence in the ClpX regulatory network in *B. anthracis.*

Acknowledgements

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