Role of the ClpXP protease in antibiotic resistance in *B. anthracis* and *S. aureus*

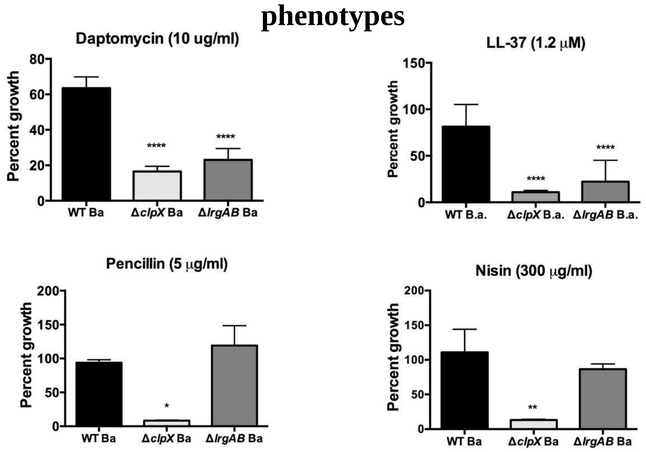
Madeline Bush, Kevin Claunch, Jacob Malmquist, Chris Evans, Shauna McGillivray
Department of Biology, Texas Christian University

Introduction

ClpX is a regulatory ATPase that functions along with ClpP as part of the intracellular bacterial ClpXP protease. ClpXP acts a global regulator that controls the lifespan of transcription factors and degrades damaged enzymes·. This protease is necessary for virulence in *Bacillus anthracis*, as well as a number of other pathogens[1]. Our previous work has shown that *B. anthracis* Sterne with a genetic deletion of ClpX (ΔClpX) is more susceptible to antimicrobial agents that target or interact with the cell wall such as daptomycin, penicillin, and LL-37[2]. In order to gain a better understanding of ClpX function in *B. anthracis*, a microarray analysis comparing WT and ΔClpX gene expression was performed and we found that LrgA and LrgB are significantly down regulated in the ΔClpX mutant. LrgA and LrgB are organized as a two gene operon, LrgAB, that has been linked to penicillin sensitivity and autolytic activity in Staphylococcus aureus[3]. We believe that loss of ClpX is leading to decreased LrgAB expression and this loss of LrgAB is contributing to increased susceptibility to cell envelope interacting antimicrobials. To test this hypothesis, we constructed a genetic deletion of LrgAB in *B. anthracis* Sterne and compared its phenotype with that of ΔClpX *B. anthracis* Sterne. We also investigated the role of ClpX and LrgAB in another gram-positive pathogen, *S. aureus*.

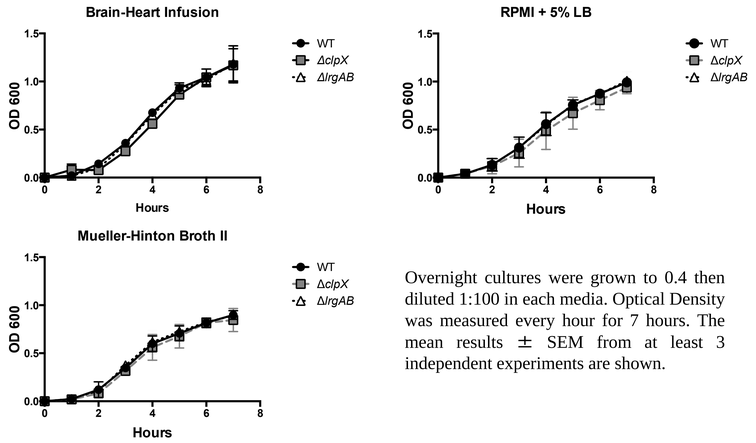
Role of ClpX and LrgAB in *B. anthracis*

ΔClpX and ΔLrgAB exhibit similar but not identical phenotypes



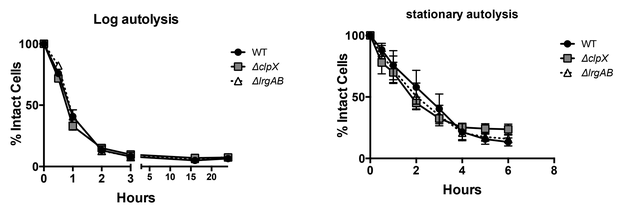
Bacterial strains were grown to log phase and incubated with the indicated antimicrobial. Optical density was measured after overnight growth and normalized to the untreated control. The mean results ± SEM from at least 3 independent experiments are shown. *, p<0.05, **, p<0.01, and ***, p<0.001 by one way ANOVA followed by Tukey's post-hoc test.

No growth differences observed in B. anthracis



Overnight cultures were grown to 0.4 then diluted 1:100 in each media. Optical Density was measured every hour for 7 hours. The mean results ± SEM from at least 3 independent experiments are shown.

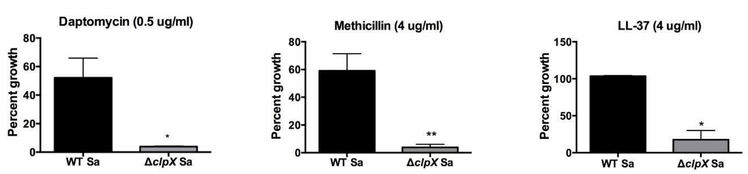
No difference in autolysis detected in *B. anthracis*



Log or stationary phase cultures were treated with .05% Triton. Results were normalized to the 0 hr time point, and the mean results ± SEM from at least 3 separate experiments are shown.

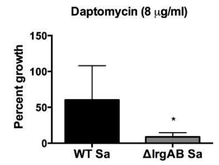
Role of ClpX and LrgAB in *S. aureus*

ClpX is necessary for antimicrobial resistance in *S. aureus*



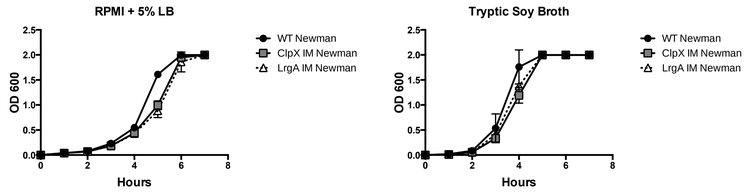
Bacterial strains were grown to log phase and incubated with the indicated antimicrobial. Optical density was measured after overnight growth and normalized to the untreated control. The mean results ± SEM from at least 2 independent experiments are shown. *, p<0.05 and **, p<0.01 by unpaired T-test.

LrgAB is necessary for daptomycin resistance in *S. aureus*



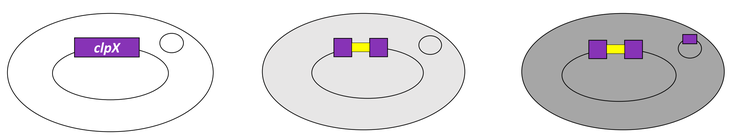
Bacterial strains were grown to log phase and incubated with daptomycin. Optical density was measured after overnight growth and normalized to the untreated control. The mean results ± SEM from at least 3 independent experiments are shown. *, p<0.05 by unpaired T-test.

No growth differences observed in *S. aureus*



Overnight cultures were grown to 0.4 then diluted 1:100 in each media. Optical Density was measured every hour for 7 hours. The mean results ± SEM from at least 3 independent experiments are shown.

Complementing ΔClpX in *S. aureus*



Conclusions

Loss of ClpX increases susceptibility to cell wall interacting antimicrobials in two pathogens, *B. anthracis* and *S. aureus*. It also results in decreased expression of LrgAB, which we show is also necessary for antimicrobial resistance in *B. anthracis* and *S. aureus*. We believe that ClpX plays a role in regulation of LrgAB however, we are still investigating the mechanism behind this regualtion. Future research will be directed towards further investigating the link between ClpX and LrgAB to better understand their connection.

Acknowledgements: Funding for this project was provided by a TCU SERC grant to Madeline Bush and TCU RCAF funds to S. McGillivray

1. McGillivray S. M., C. M. Ebrahimi, N. Fisher, M. Sabet, D. X. Zhang, Y. Chen, N. M. Haste, R. V. Aroian, R. L. Gallo, D. G. Guiney, A. M. Friedlander, T. M. Koehler, and V. Nizet. 2009. ClpX contributes to innate defense peptide resistance and virulence phenotypes of Bacillus anthracis. J Innate Immun 1:494-506
2. McGillivray S. M., D. N. Tran, N. S. Ramadoss, J. N. Alumasa, C. Y. Okumura, G. Sakoulas, M. M. Vaughn, D. X. Zhang, K. C. Keiler, and V. Nizet. 2012. Pharmacological inhibition of the ClpXP protease increases bacterial susceptibility to host cathelicidin antimicrobial peptides and cell envelope-active antibiotics. Antimicrob Agents Chemother 56:1854-1861.
3. Groicher . H., . A. Firek, . F. Fujimoto, and . W. Bayles. 2000. The Staphylococcus aureus lrgAB Operon Modulates Murein Hydrolase Activity and Penicillin Tolerance. Journal of Bacteriology 182:1794-1801.