Investigating the role of fused *msrA/B* and *clpX* in the resistance to cell-wall antibiotics in *Bacillus anthracis* Sterne

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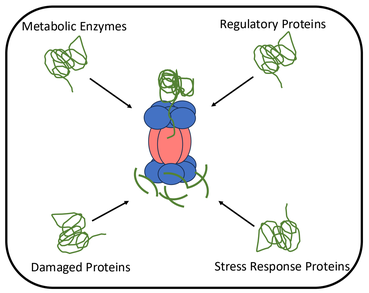
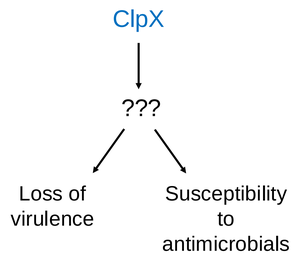
Background

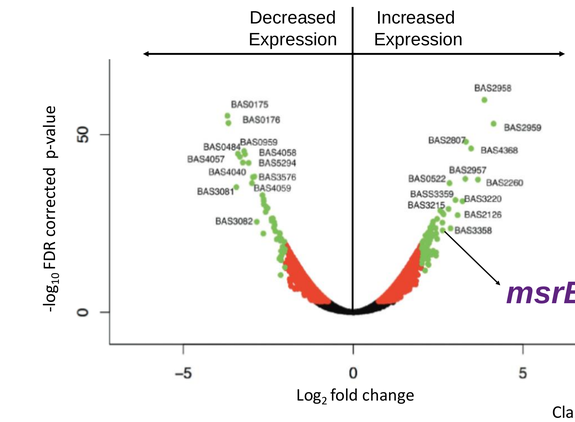
ClpX is essential for virulence in *Bacillus anthracis* and critical for resistance to cell envelope-targeting antimicrobials. However, the exact mechanism behind this phenomenon is not yet fully understood. ClpX is a regulatory subunit of a major global protease, ClpXP.





ClpX: recognizes and unfolds proteins
ClpP: degrades proteins

Differential Gene Expression in Δ*clpX*

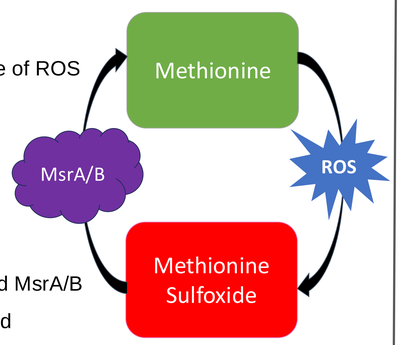


Claunch et al., 2018

Methionine Sulfoxide Reductase (MsrA and MsrB)

Methionine Sulfoxide Reductase:

- Antioxidant enzyme; linked to the tolerance of ROS
- Can exist independently or fused



In *B. anthracis*:

- No previous connection between ClpX and MsrA/B
- The role of MsrB has not been investigated

Fused MsrA/B:

- At least 30 species of bacteria possess a fused msrA/B
  - Notably: *Streptococcus pneumonia, Helicobacter pylori* and *Neisseria* spp.
- Fused MsrA/B possess similar enzymatic kinetics are independent units
  - In some species the MsrB subunit is more enzymatically active

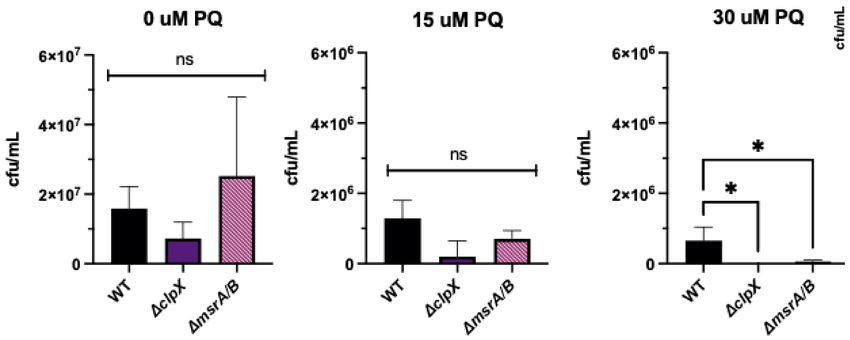
Antibiotic MIC Table

Strains	Penicillin (μg/mL)	Daptomycin (μg/mL)	LL-37 (μg/mL)
WT	80	16	2
Δ*clpX*	**1.25**	**8**	**1**
Δ*msrA/B*	**20**	16	2
	Cell wall	Cell membrane	

Susceptibility to ROS

ROS	H_2O_2 (%)	Bleach (%)	Paraquat (μM)
WT	0.008	0.15	60
Δ*clpX*	**0.004**	0.15	**30**
Δ*msrA/B*	0.008	0.15	**30**

Survival in Paraquat (PQ)

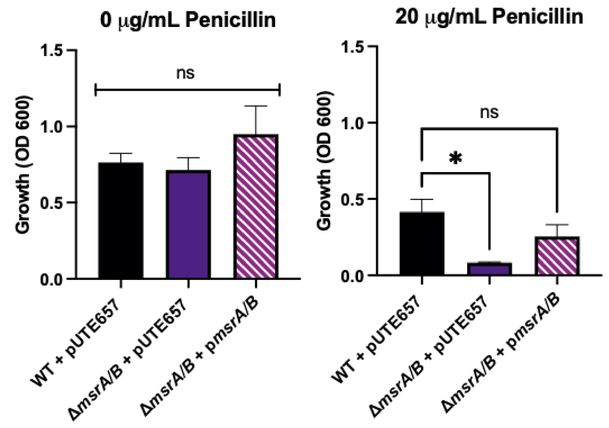


Data is represented as mean +/- SD of at least four independent experiments. ns indicates a non-significant difference, and * represents a p-value < 0.05, as determined by one-way ANOVA, followed by Tukey-Kramer *post hoc* analysis.

Future Directions

- Examine the regulation of *msrA/B* expression in WT and Δ*clpX* with and without penicillin and daptomycin
- Construct a complete knockout of *msrA/B* in WT *B. anthracis* Sterne
- Mutate critical cysteines in the active sites of the fused *msrA/B* for future assays

Complementation with Δ*msrA/B*



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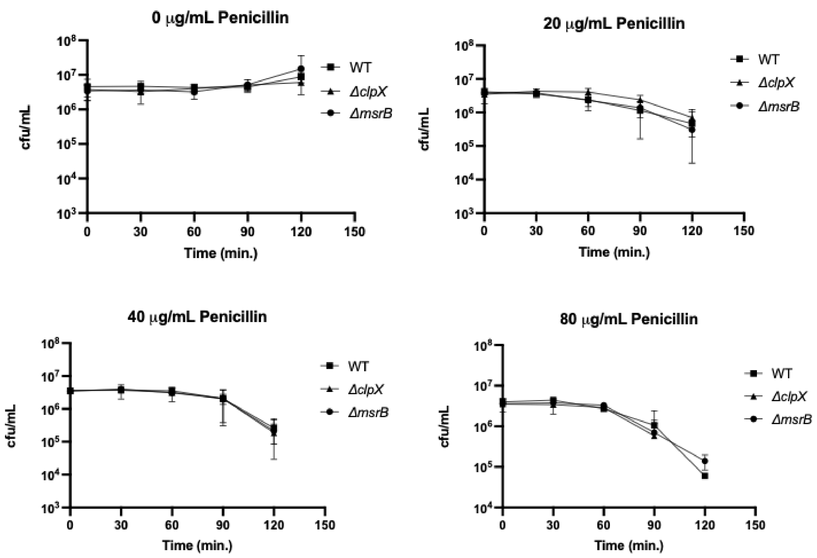
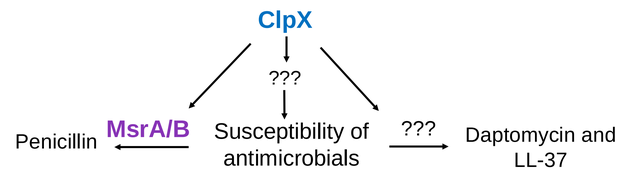
survival Curve



Summary



Acknowledgments

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