



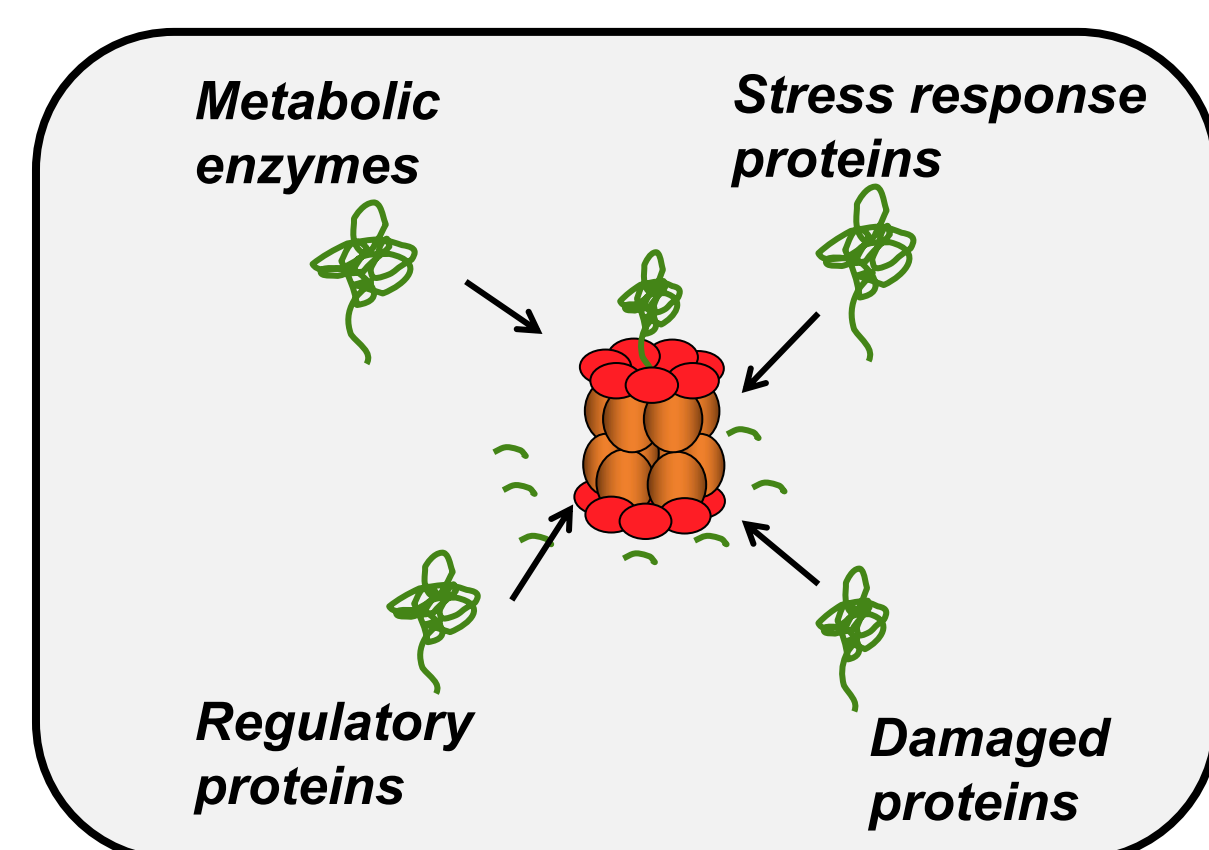
Regulatory effects of loss of ClpX on the *msrA* chromosomal gene in *Bacillus anthracis*

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Introduction

Bacillus anthracis is the causative agent of anthrax. Previously, our lab identified the *clpX* gene as critical for virulence in *B. anthracis*. The $\Delta clpX$ mutant exhibited decreased cell wall integrity and increased susceptibility to cell-envelope active antibiotics. ClpX is one component of the intracellular caseinolytic protease ClpXP that degrades multiple proteins including transcriptional regulators. To understand changes in gene expression in $\Delta clpX$, a microarray comparing WT and $\Delta clpX$ was conducted. 100+ genes were identified as significantly differentiated in the $\Delta clpX$ mutant. This project focuses on *msrA*, an upregulated gene in $\Delta clpX$. MsrA is an antioxidant enzyme that reduces methionine-S-sulfoxide to methionine and is highly induced with oxacillin exposure in *S. aureus*². The goal of this study is to determine if loss of the *msrA* gene impacts antibiotic susceptibility. We hypothesized that since *msrA* is upregulated in $\Delta clpX$, $\Delta msrA$ would exhibit the opposite phenotype. This research is important as it aids our understanding of bacterial defenses and may provide new drug targets to help combat rising antibiotic resistance.

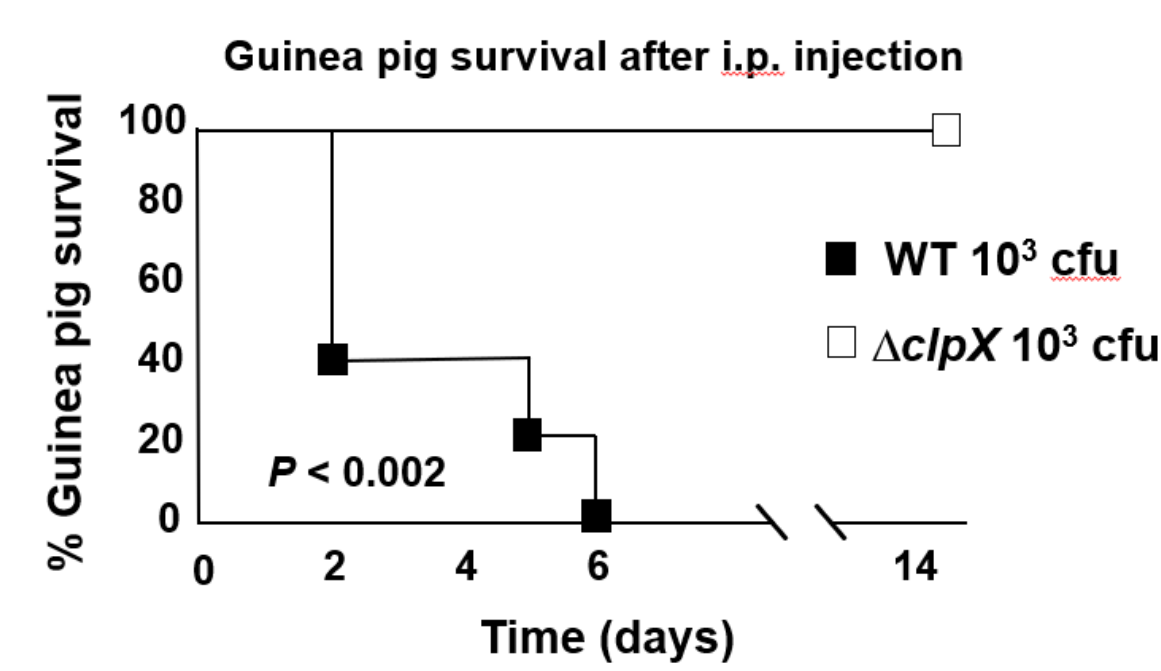
ClpX role in ClpXP caseinolytic protease



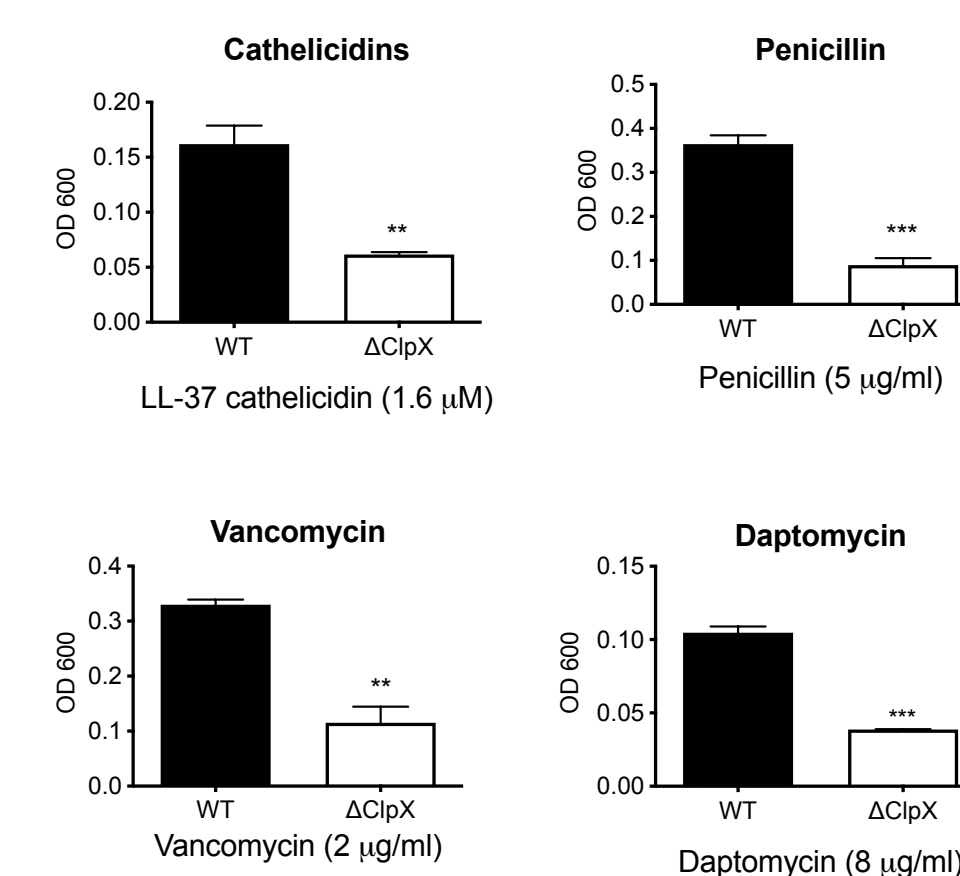
ClpX: regulatory ATPase
Recognizes and unfolds proteins

ClpP: proteolytic core
Degrades proteins

ClpX is necessary for virulence and antibiotic resistance

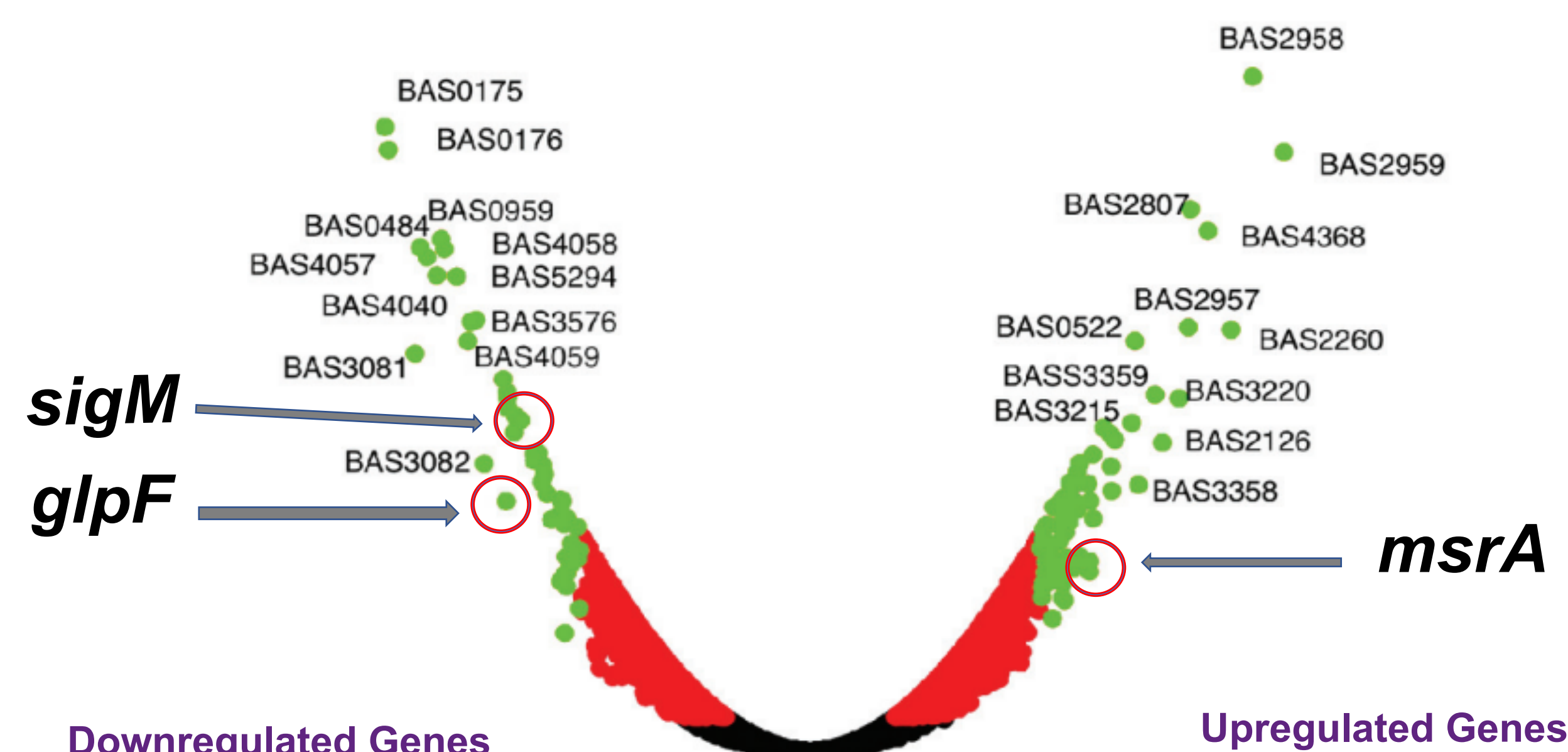


Adapted from McGillivray et al 2009



Adapted from Zou et al 2021

Volcano plot reveals differentially regulated genes in $\Delta clpX$

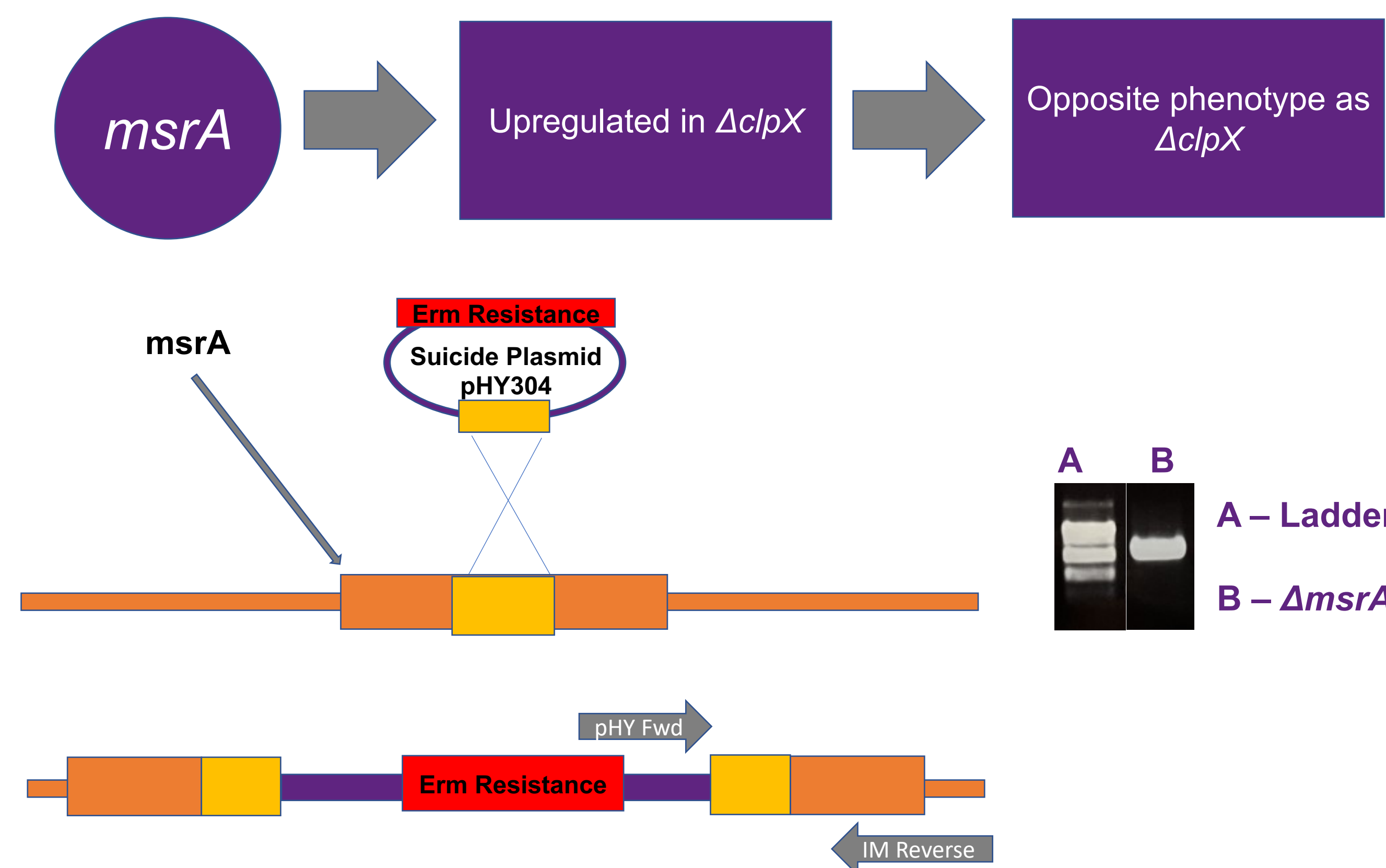


Downregulated Genes

Upregulated Genes

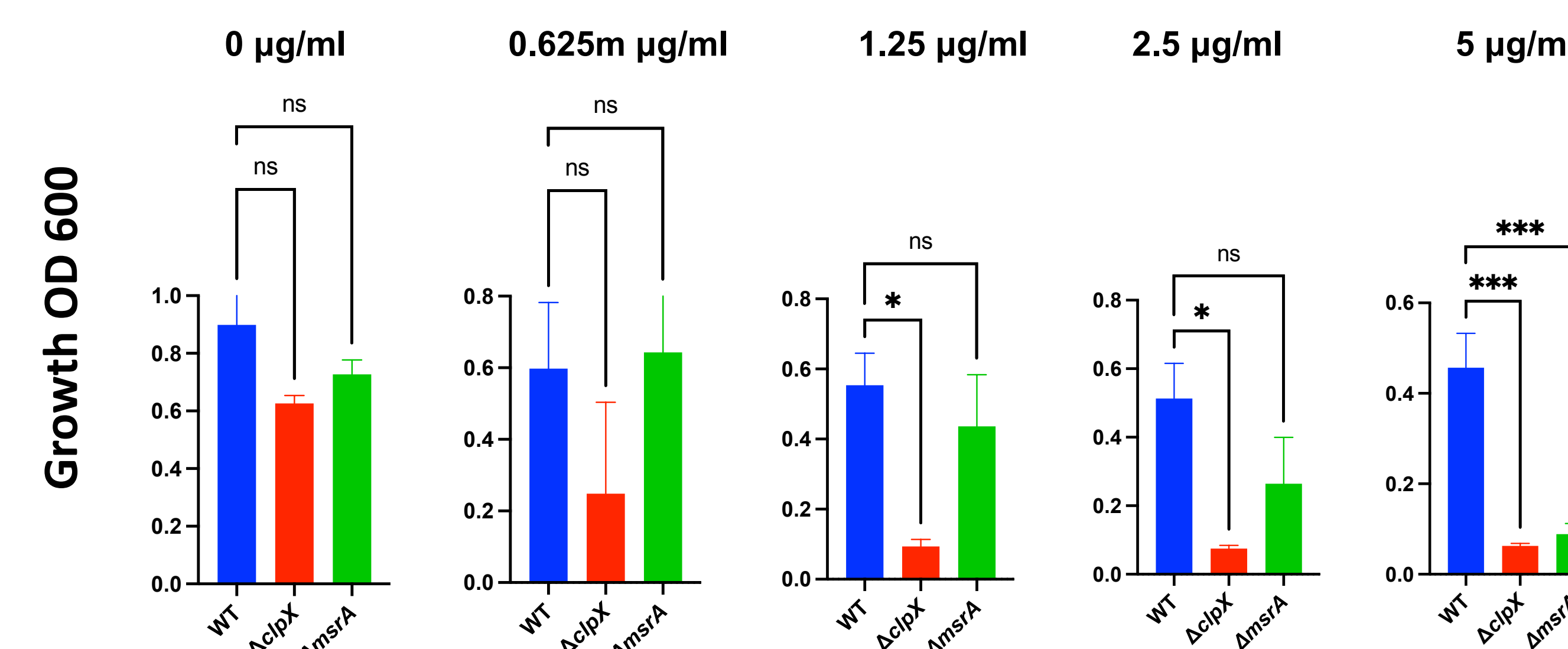
Adapted from Claunch et al 2018

Hypothesis

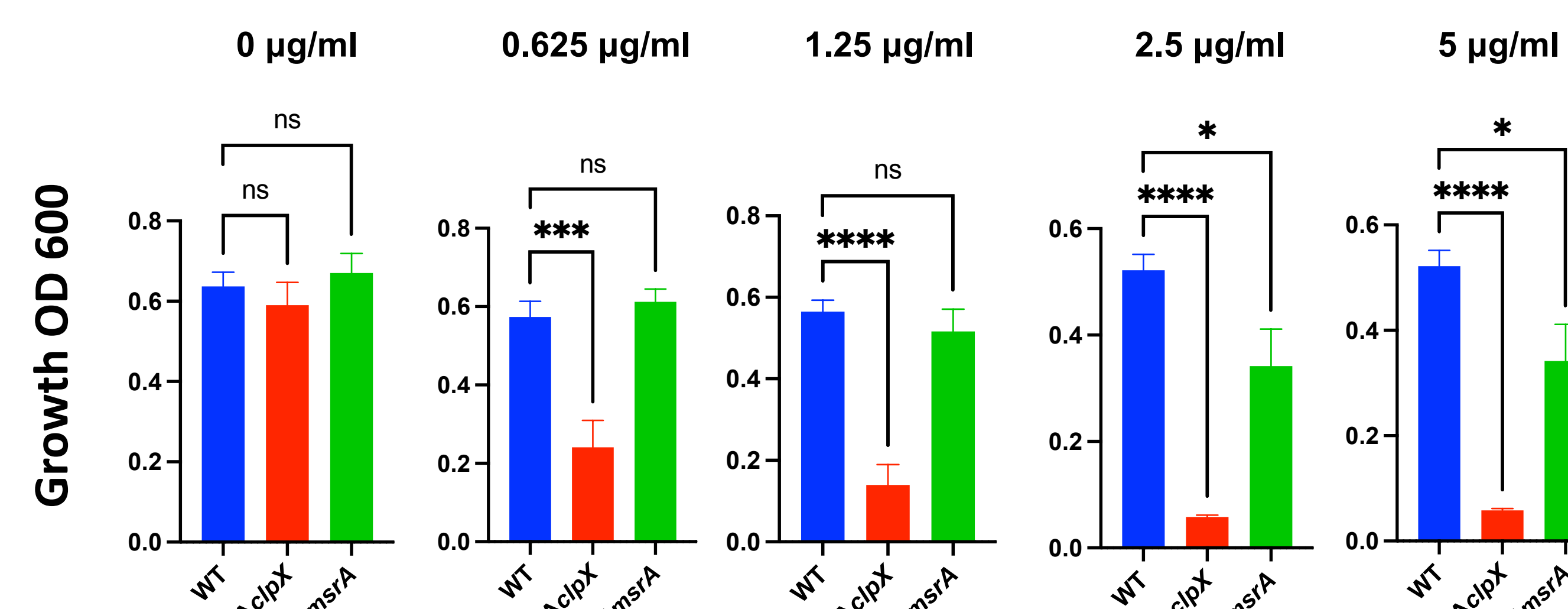


$\Delta msrA$ susceptible to penicillin

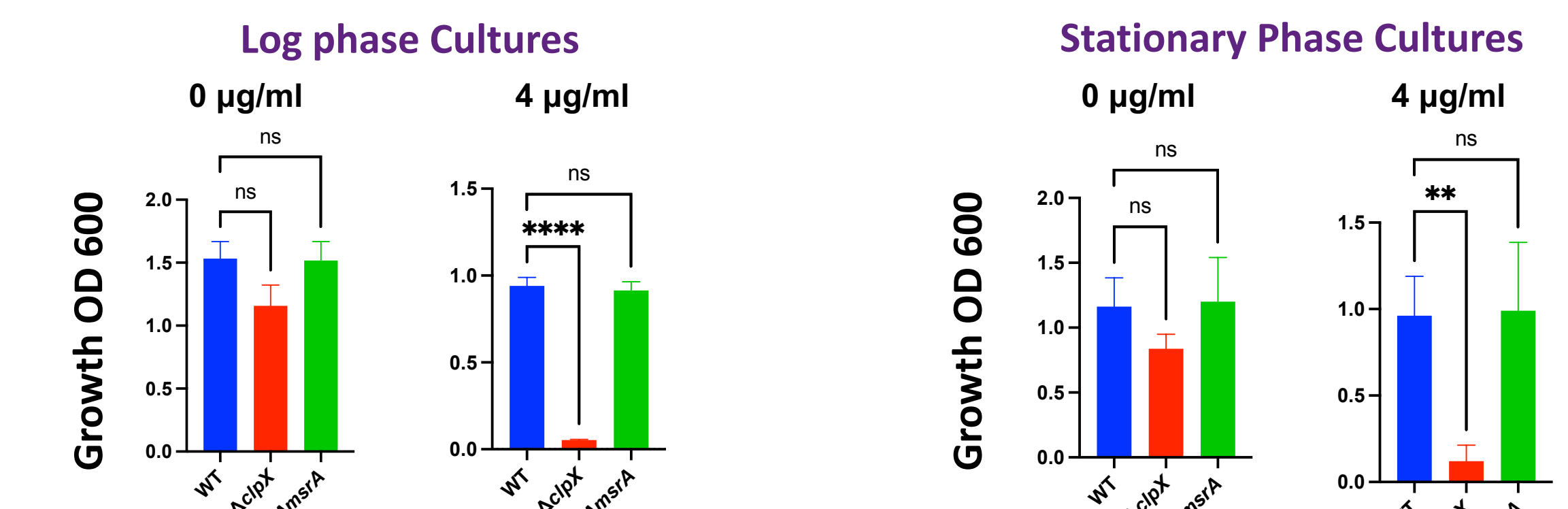
Log Phase Cultures



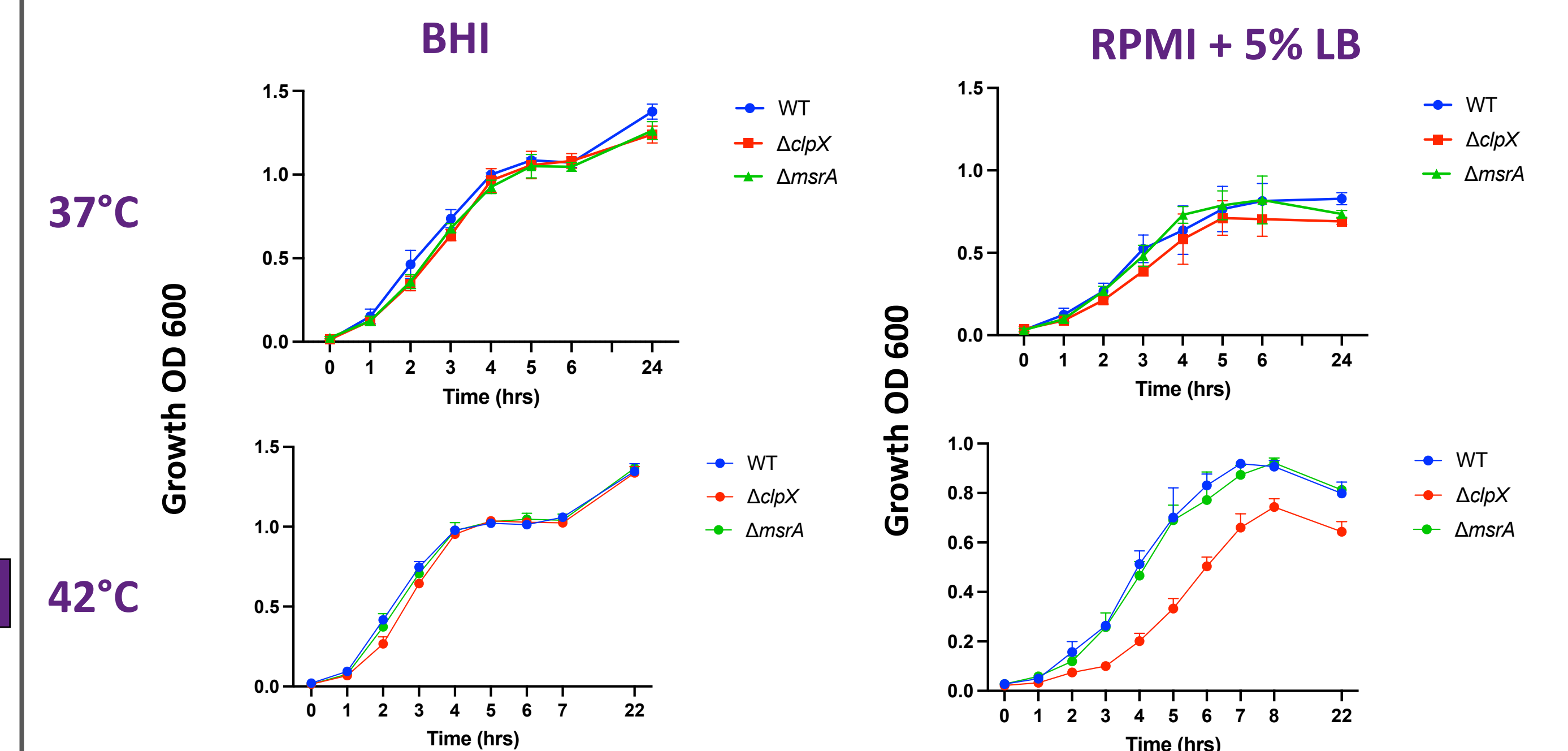
Stationary Phase Cultures



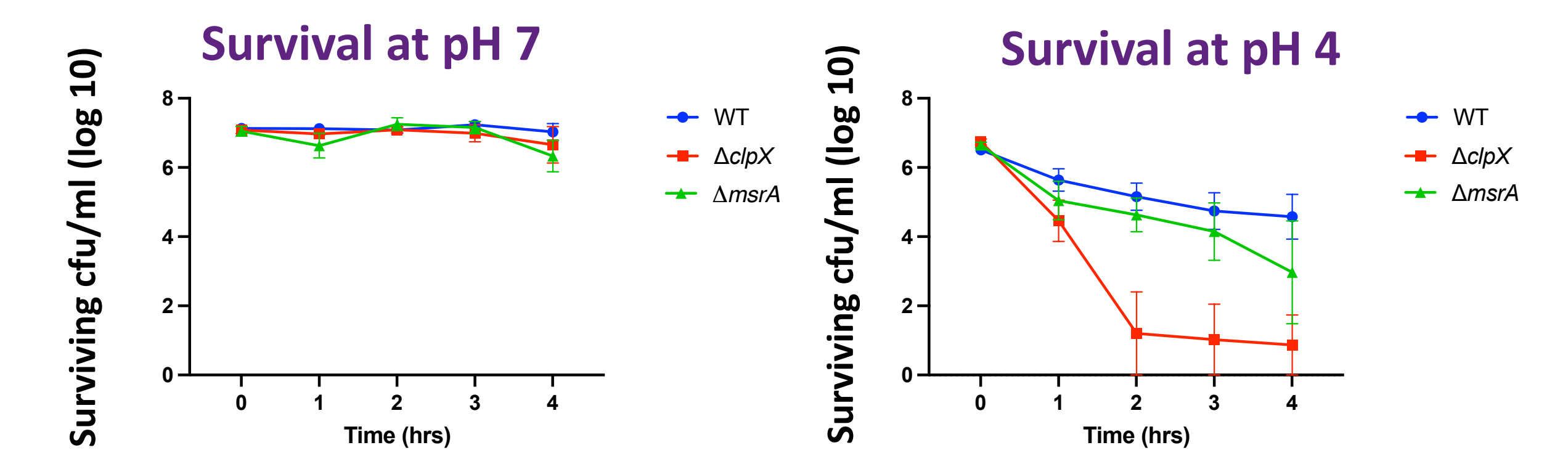
$\Delta msrA$ does not show sensitivity with daptomycin



$\Delta msrA$ does not show sensitivity with heat stress



$\Delta msrA$ does not show sensitivity with acid stress



Conclusions & Future Directions

We hypothesized that since $\Delta msrA$ is upregulated in $\Delta clpX$, $\Delta msrA$ would exhibit the opposite phenotype. Surprisingly, we find that $\Delta msrA$ has significant growth inhibition in the presence of penicillin. This data corresponds with another study that found upregulation of *msrA* in the presence of the cell wall active antibiotic oxacillin in *S. aureus*³. At this time, there is no clear answer as to why MsrA is critical in antibiotic response. We do not find susceptibility to other antibiotics, such as daptomycin, nor does it appear to be more susceptible to other ClpX-related stress responses such as heat or acid stress. Future research will test $\Delta msrA$ susceptibility to additional antimicrobials, such as the antimicrobial peptide LL-37 and the antibiotic vancomycin, as well as $\Delta msrA$ virulence *in vivo* with the *Galleria mellonella* infection model. We will also complement $\Delta msrA$ to confirm the phenotypes are due to loss of the *msrA* gene.

Acknowledgements



Funding for this project was provided by TCU SERC grants to Kelsey Waite
TCU Biology Department

References

1. Claunch, K.M., et al., *Transcriptional profiling of the clpX mutant in Bacillus anthracis reveals regulatory connection with the IrgA operon*. Microbiology (Reading), 2018, 164(4): p. 659-669.
2. Singh, V.K., et al., *Molecular characterization of a chromosomal locus in Staphylococcus aureus that contributes to oxidative defence and is highly induced by the cell-wall-active antibiotic oxacillin*. Microbiology-Sgm, 2001, 147: p. 3037-3045.
3. Utaida S, Dunman PM, Macapagal D, Murphy E, Projan SJ et al. *Genome-wide transcriptional profiling of the response of Staphylococcus aureus to cell-wall-active antibiotics reveals a cell-wallstress stimulon*. Microbiology 2003;149:2719-2732