



Role of ClpX in regulation of stress responses in *Bacillus anthracis*

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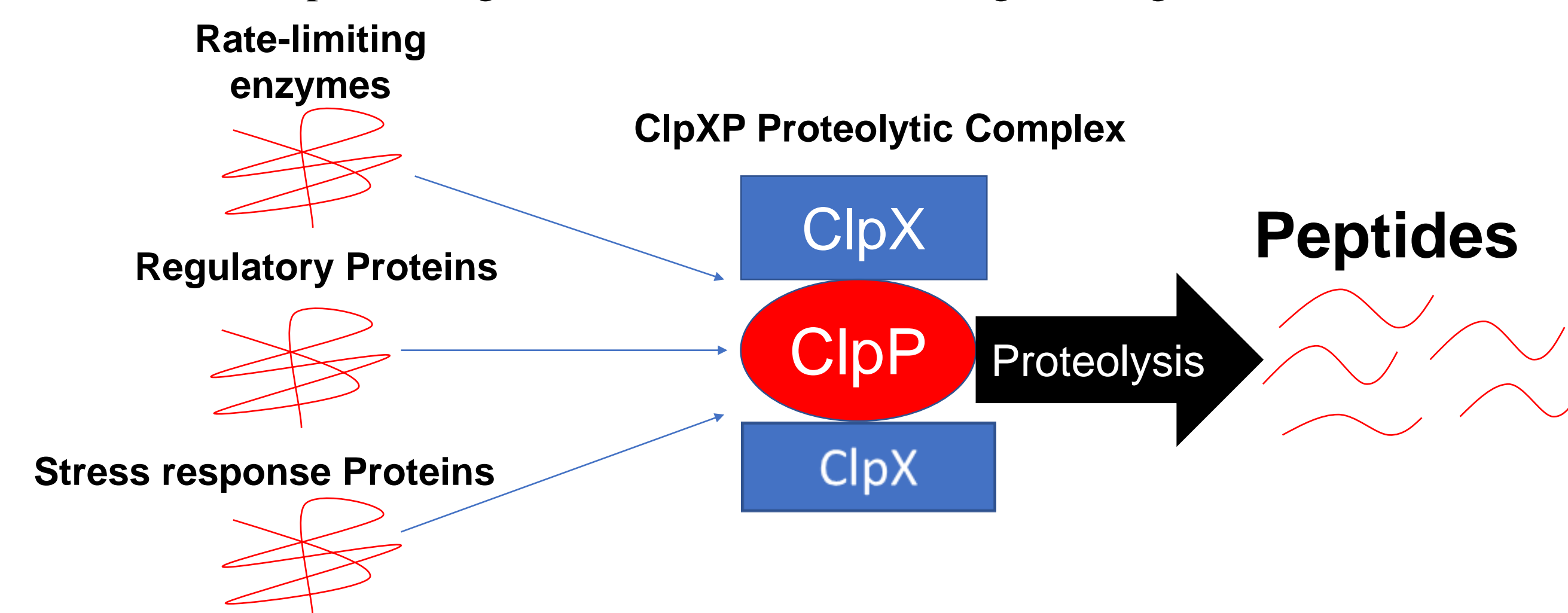
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Abstract

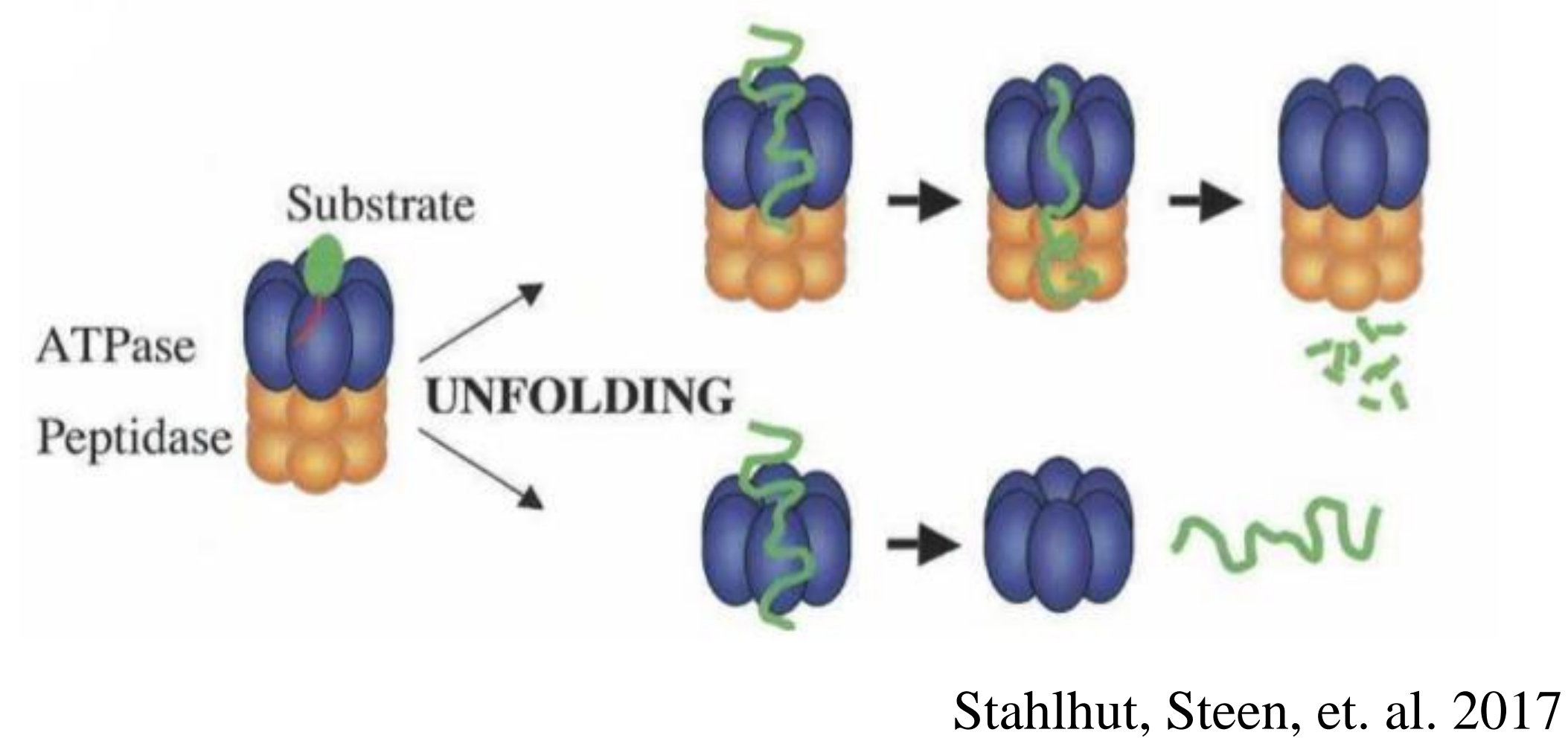
Anthrax is an infectious disease caused by *Bacillus anthracis*, which is a spore forming bacterium. Even though the anthrax toxins and capsule, encoded on 2 plasmids pXO1 and pXO2, play crucial role in the pathogenesis of anthrax infection, evidence suggests that chromosomal genes also play a role. The ClpX ATPase was discovered to be crucial for *B. anthracis* virulence via protection against host antimicrobial peptides. In this study, we want to investigate the role of *clpX* in regulation of other stressors including acidic stress, temperature stress, salt stress, and non-cell envelope active antibiotics. We found that *clpX* is necessary for survival in an acidic environment and growth under heat stress. We demonstrate that acidic stress resistance is mediated by the formation of the ClpXP protease using a ClpX complementation plasmid that is incapable of interacting with ClpP. There is no association between *clpX* with other stressors. We conclude that the ClpX is required for *B. anthracis* pathogenicity via defenses against host antimicrobial peptides and for survival in an acidic environment. Understanding the role ClpX in the regulation of stress responses will ultimately infer us with new target for either directly combating infection or improving the efficacy of already available medicines.

Background

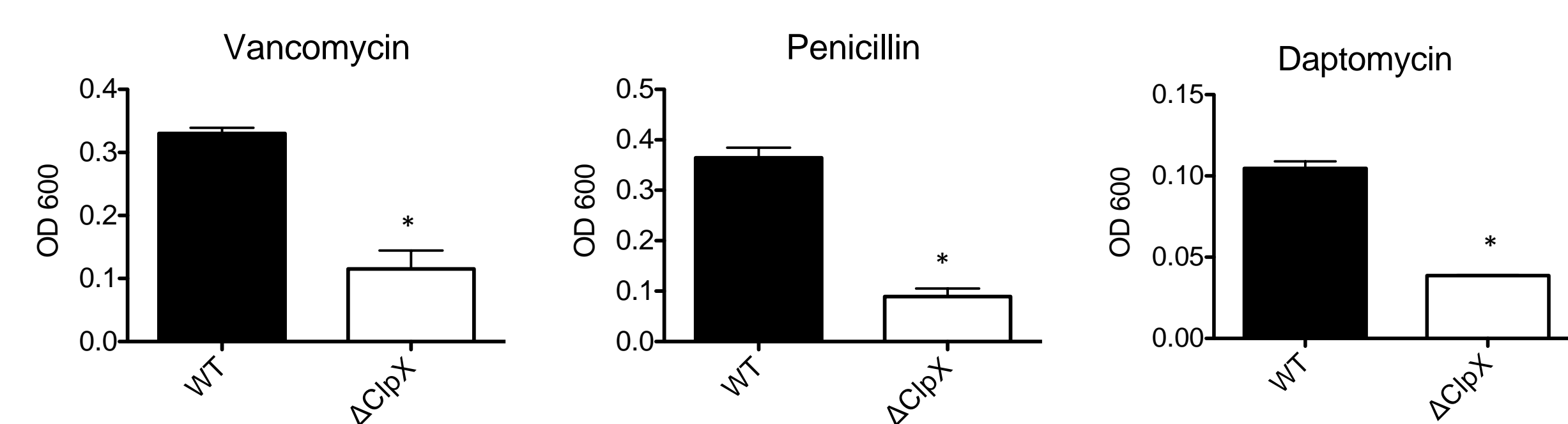
ClpX is one of several Clp ATPases that interacts with a proteolytic core called ClpP to form the Clp protease complex. ClpXP degrades a variety of targets, including rate-limiting enzymes, stress response proteins, and transcriptional regulators, and hence acts as a global regulator.



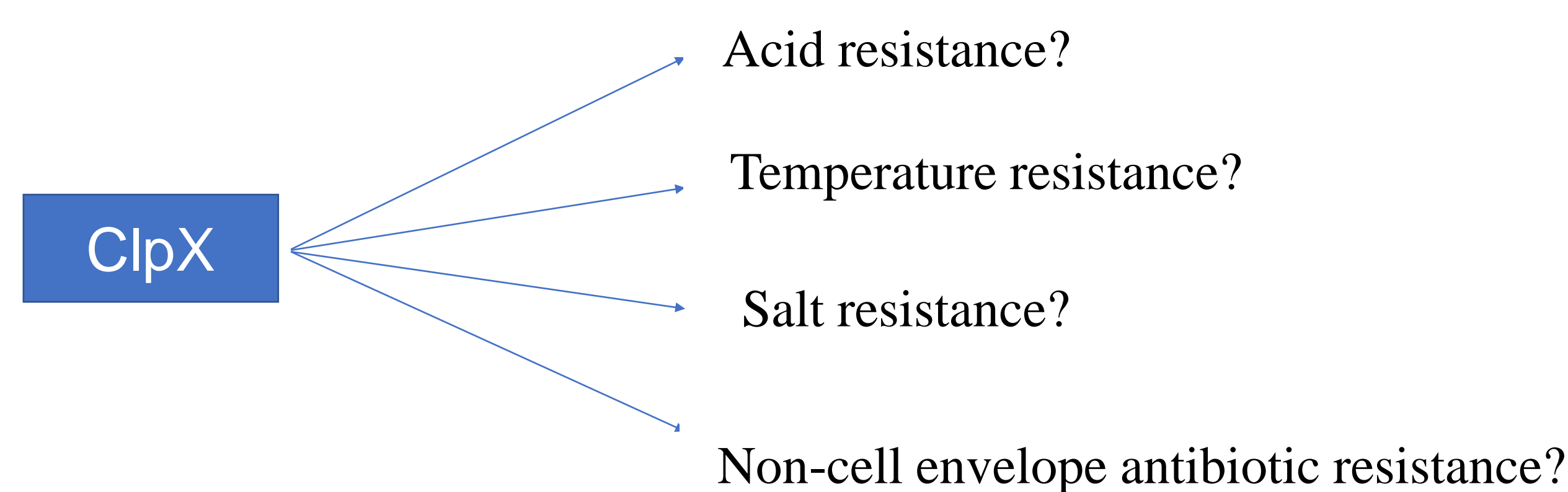
Beside protease activity, Clp ATPases can function independently as a chaperone where it takes misfolded proteins and converts them back to the correctly folded protein.



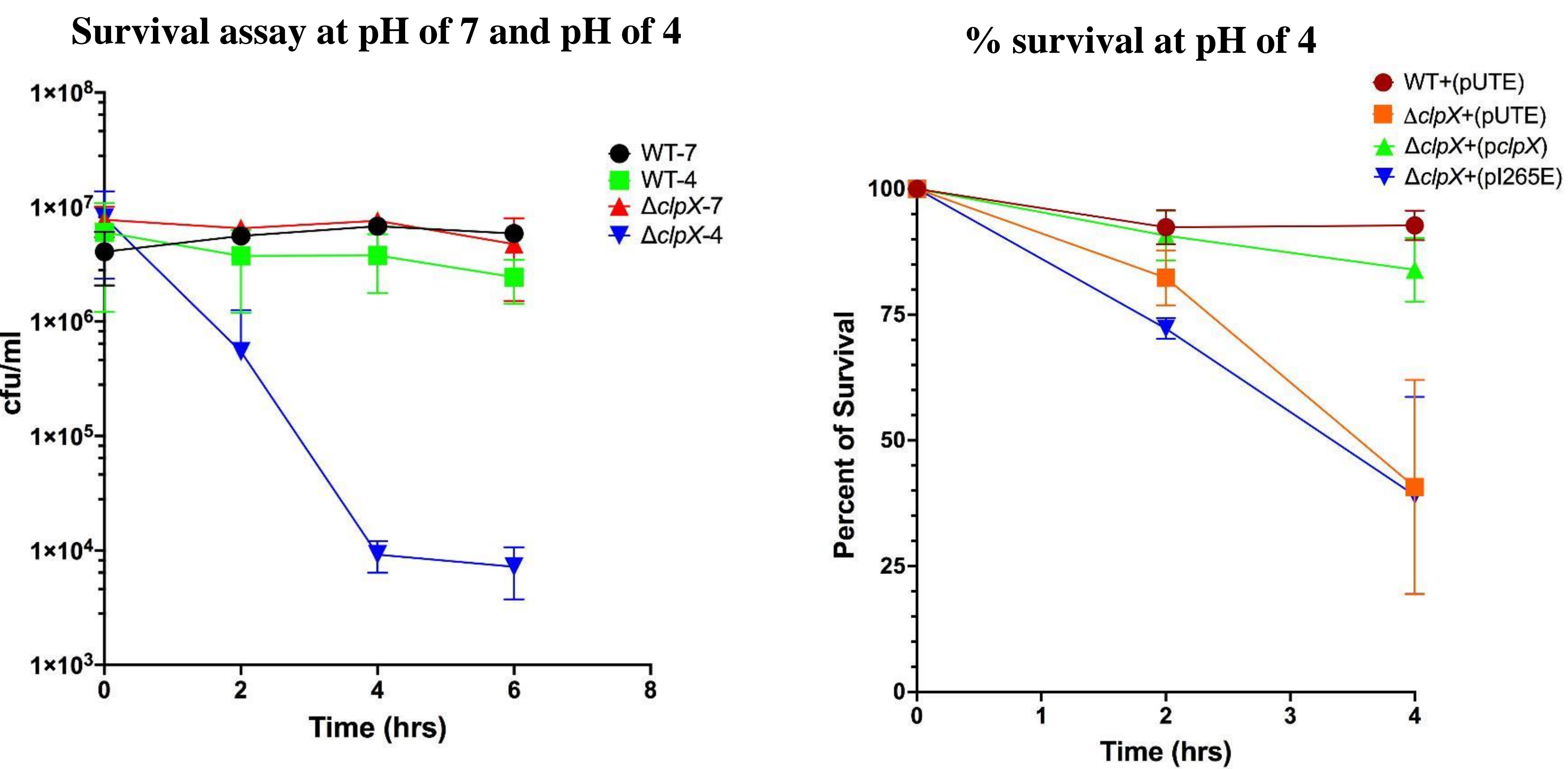
ClpX has been shown to play an important role in defense against cell-envelope targeting antibiotics.



Role of ClpX in *B. anthracis* stress response

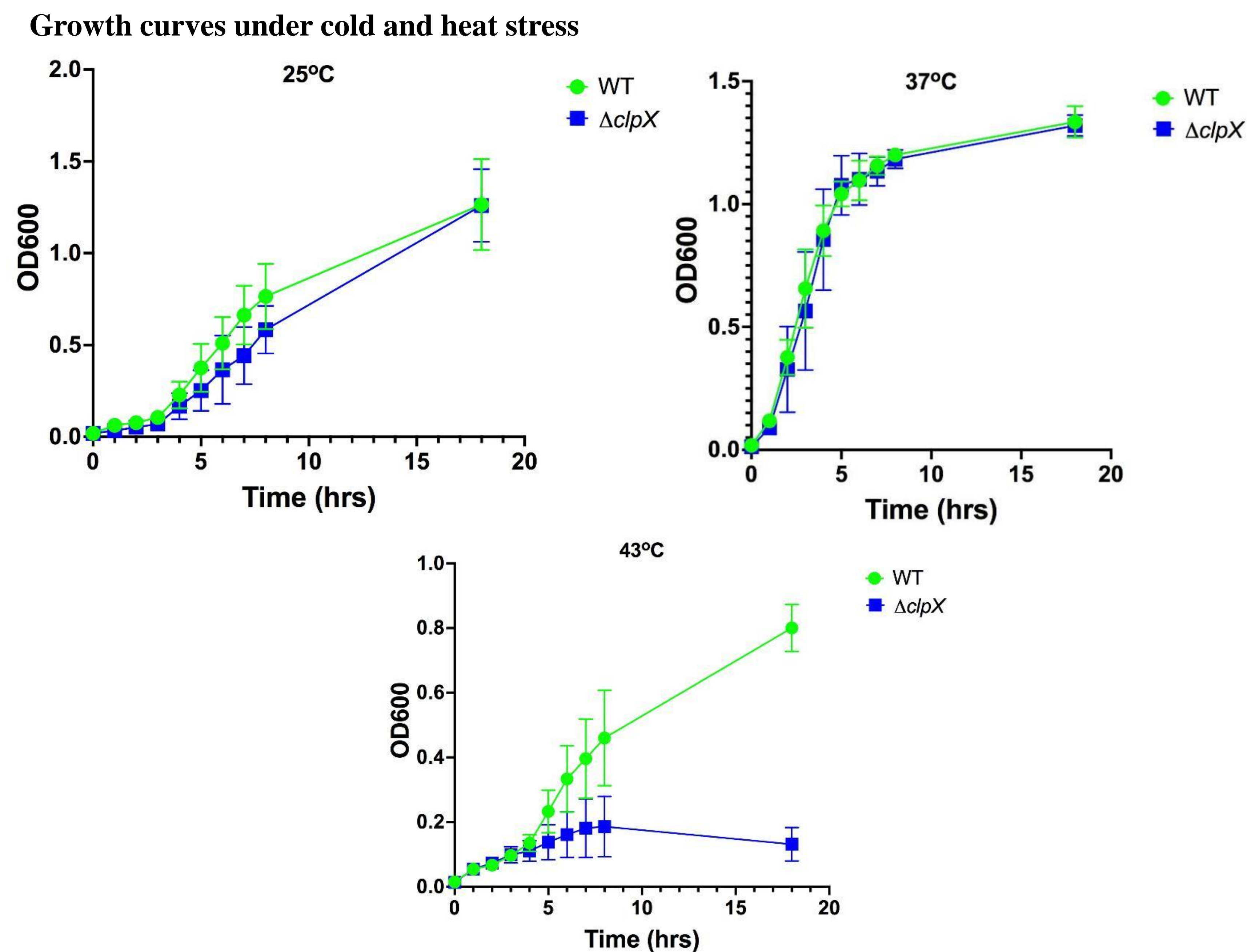


Acid Stress



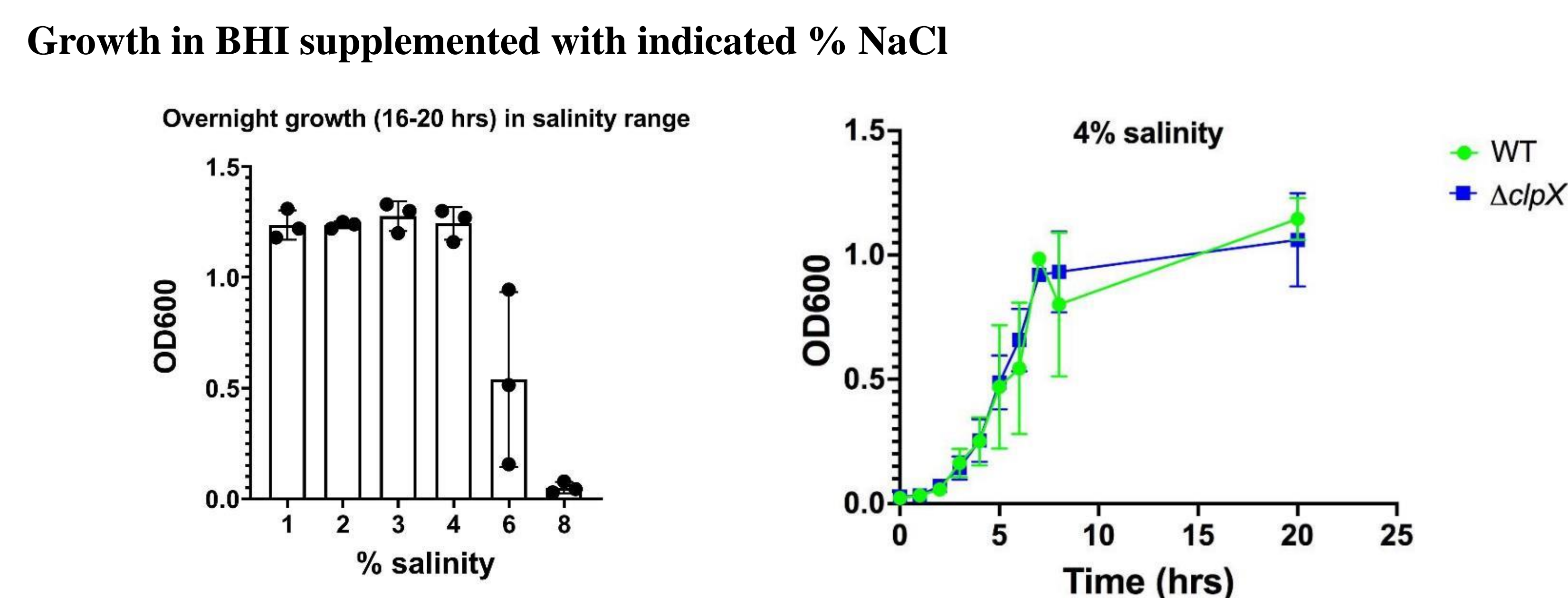
Loss of *clpX* leads to decreased survival in an acidic environment.

Temperature Stress



clpX is required for growing under the conditions of heat stress but not cold stress.

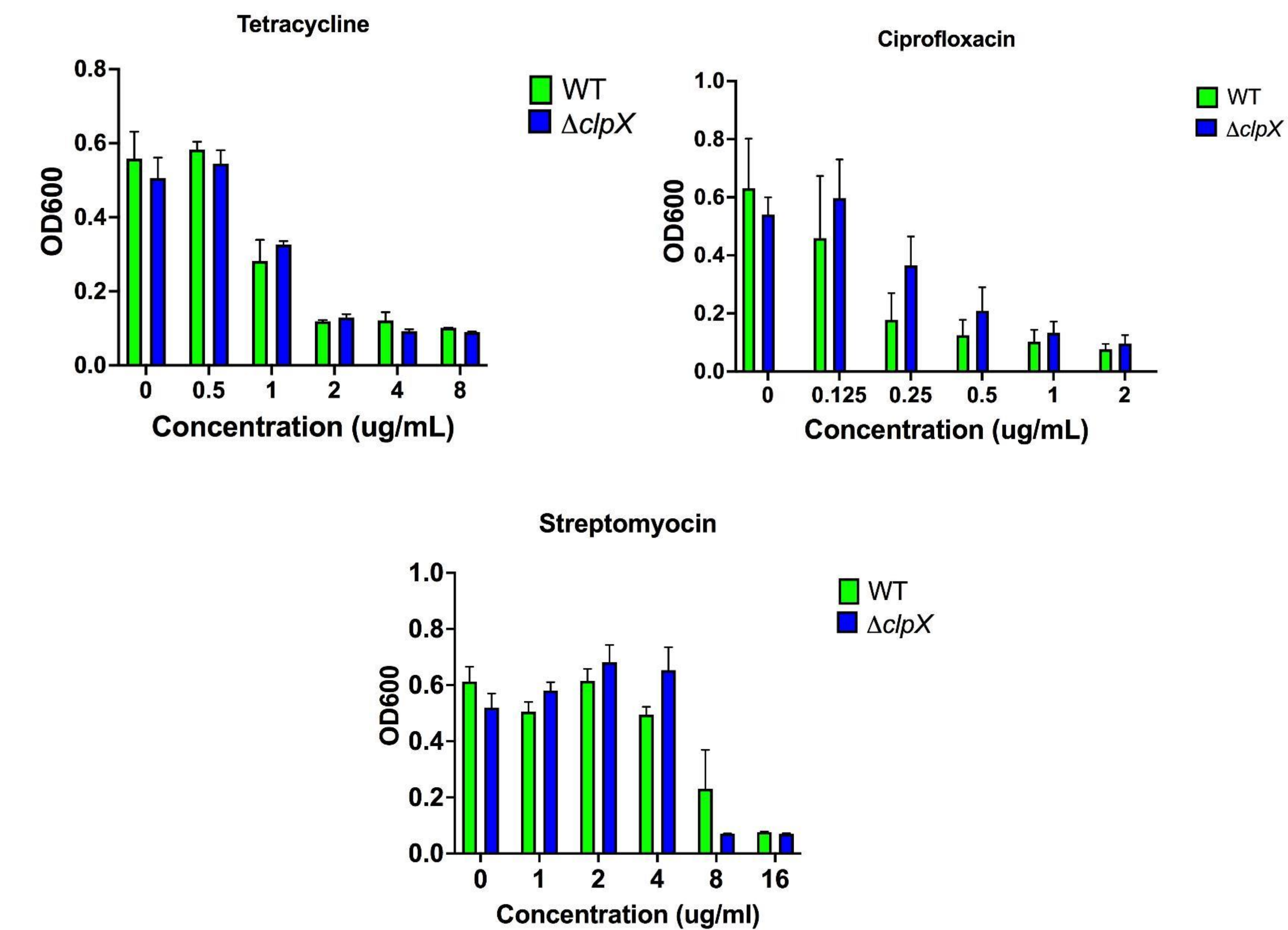
Salt stress



There is no significant difference in growth with increased salt concentrations between wild-type *B. anthracis* Sterne and $\Delta clpX$.

Antibiotic Stress

MIC assays using non-cell envelope targeting antibiotics



clpX does not contribute to the resistance of non-cell envelope targeting antibiotics.

Conclusions & Future Directions

Conclusions

- Disruption of *clpX* in *B. anthracis* Sterne leads to reduced survival in an acidic environment and growth at high temperature.
- ClpX-mediated acidic pH resistance is dependent upon the interaction with ClpP through the ClpXP protease rather than through ClpX-independent mechanisms.
- Loss of ClpX does not affect response to cold stress (25°C), salt stress and the non-cell envelope antibiotics tetracycline, ciprofloxacin, or streptomycin.

Future Directions

- Investigate the role of the other Clp ATPases found in *B. anthracis*, ClpB and ClpC, in regulation of stress responses.

Acknowledgements

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