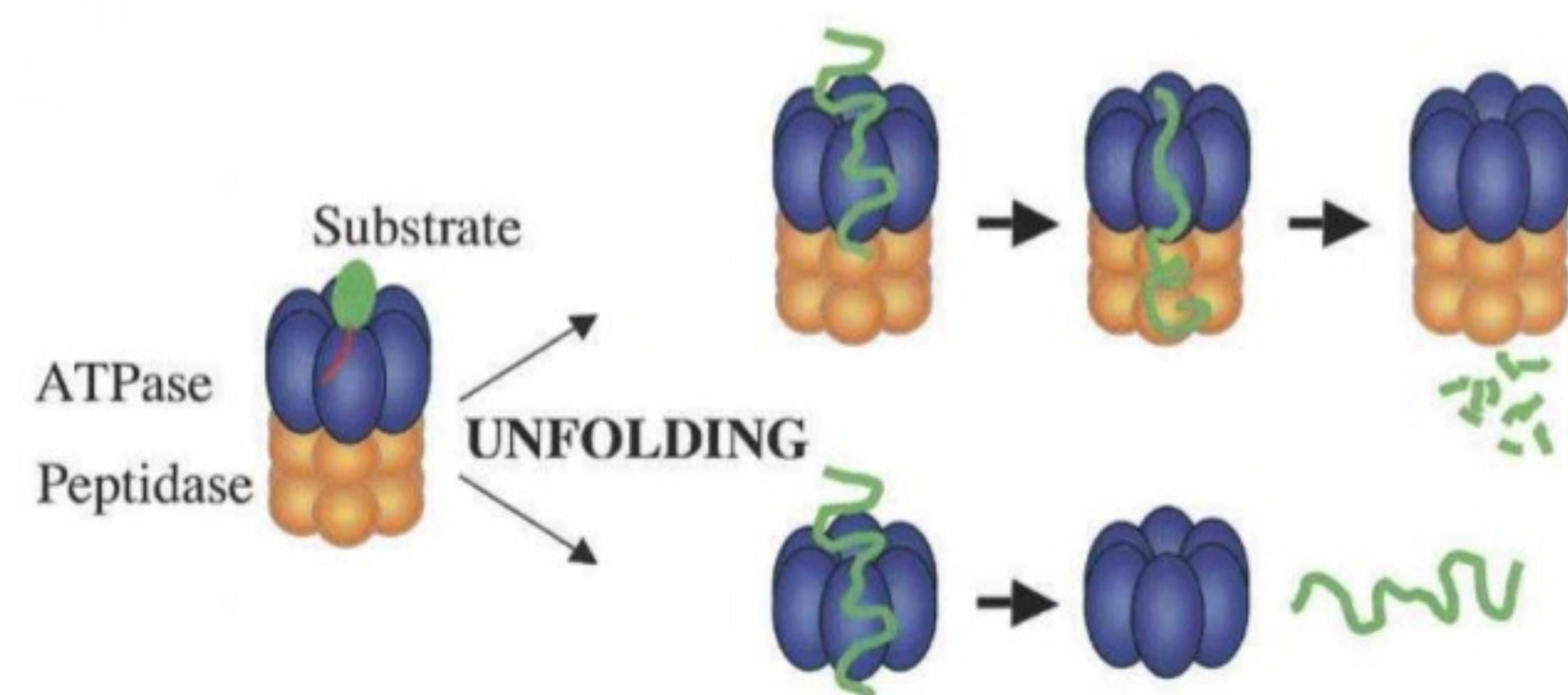


# The role of ClpX in the stress response and virulence of *Bacillus anthracis*: protease or chaperone?

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## Background

ClpX is one of several Clp-ATPases that can interact with ClpP to form a protease complex, or act alone as a chaperone.

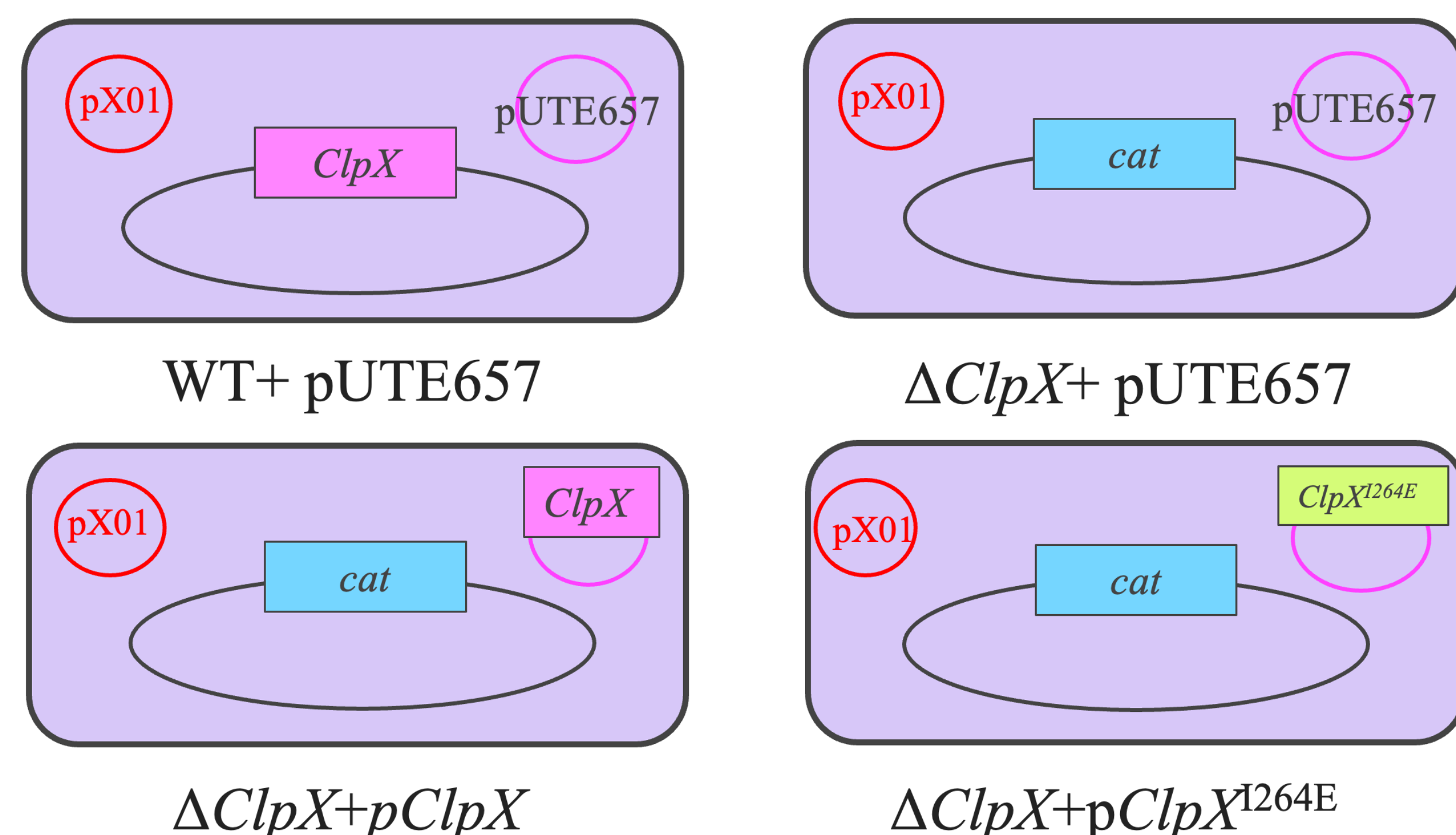


The bolded amino acids are part of a highly conserved region that is important for ClpP association, and the amino acid marked in red is the target of site-directed mutagenesis. This mutation inhibits ClpXP binding allowing only chaperone activity to take place.

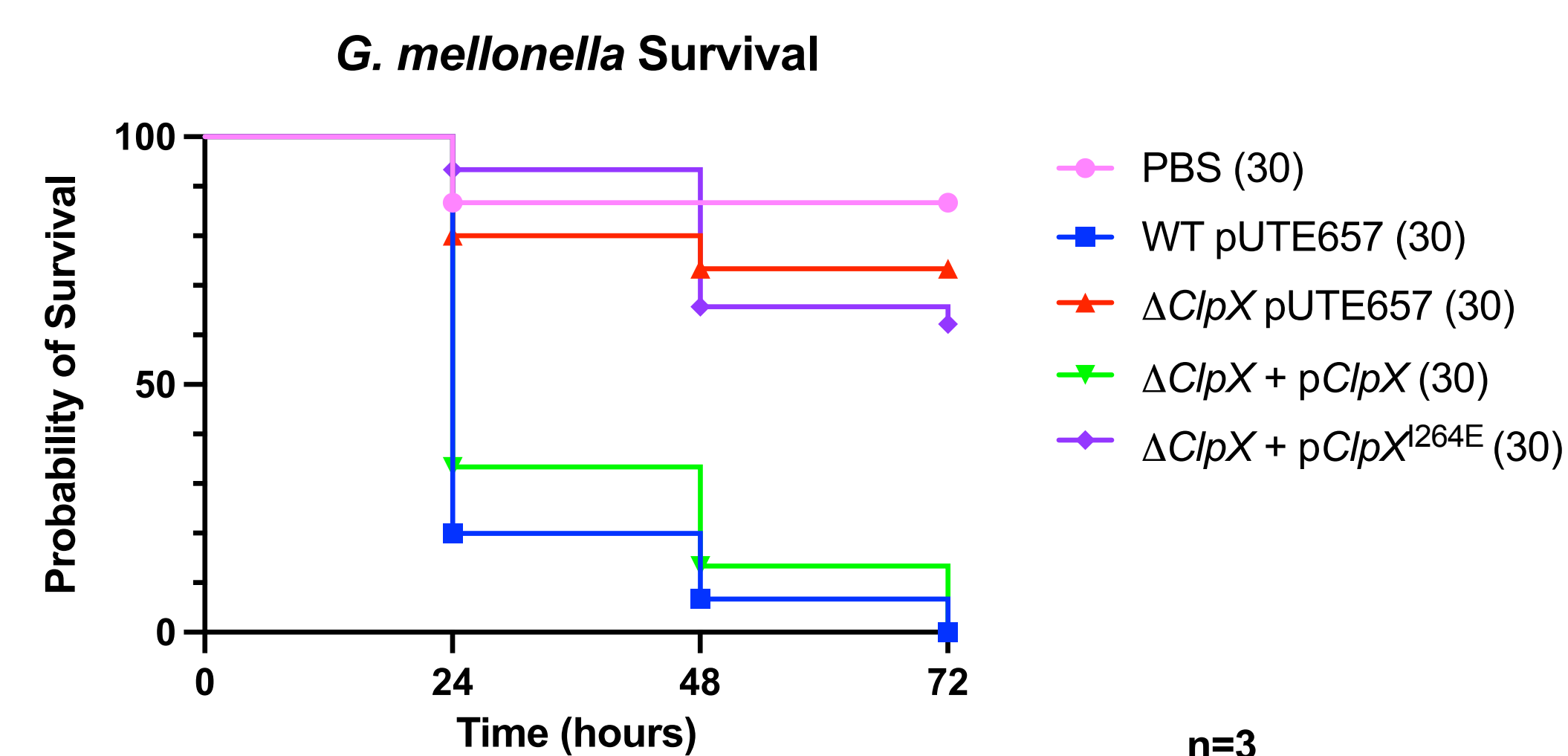
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Sa ClpX: TTNILFILGGAFDGIIEVIK RRGEKVIIGSS-NEAD KYDEQALLAQIRPEDLQAYG
Ba ClpX: TTNILFICGGAFDGIPIIK RRGEKVIIGSGSEKNA DVNEKHVLSHVLPEDDLRFG
    
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Four strains in the unencapsulated Sterne strain of *Bacillus anthracis* were created to investigate the mechanism of action of ClpX in antibiotic stress.

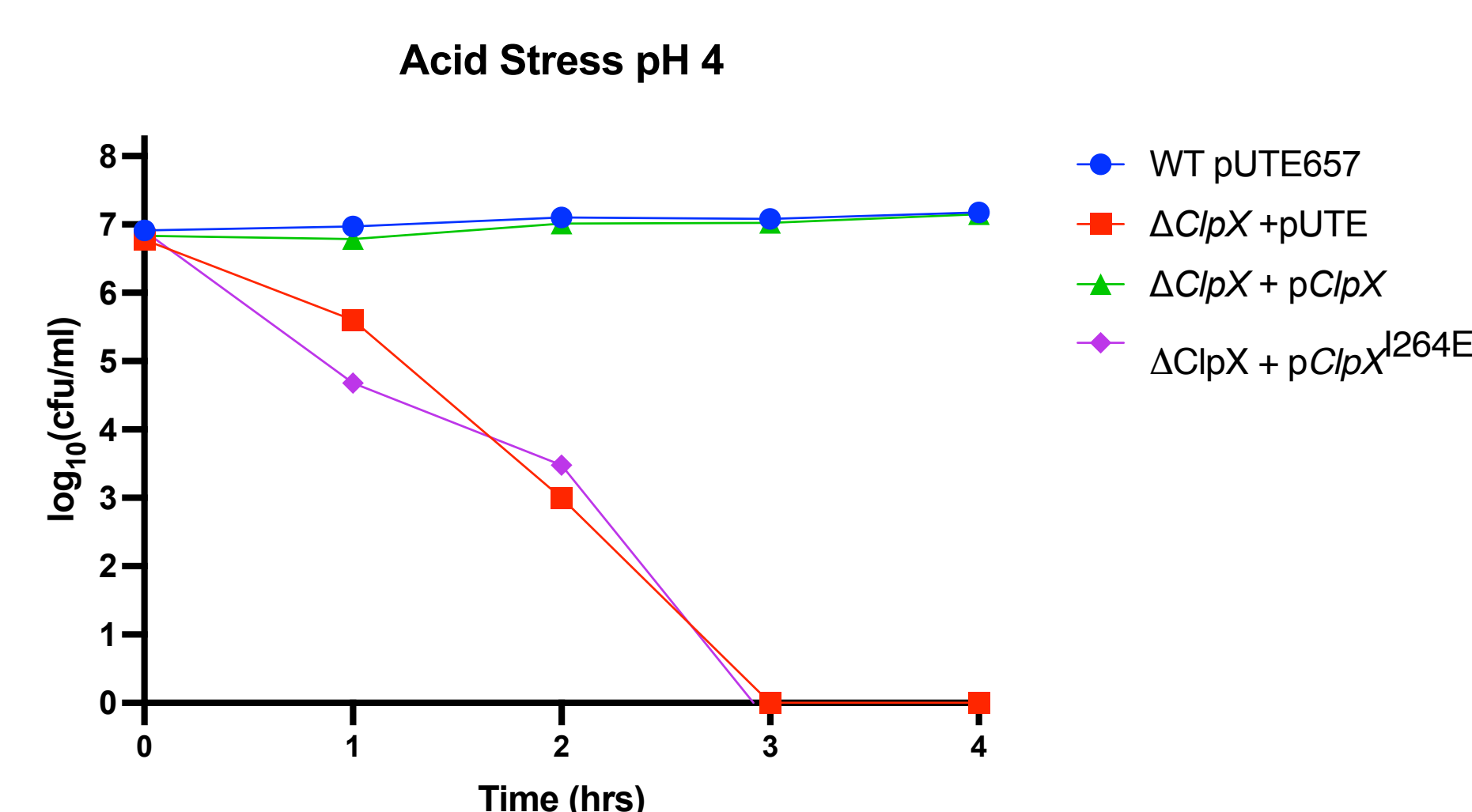


## ClpXP formation is necessary for virulence *in vivo* in *G. mellonella*

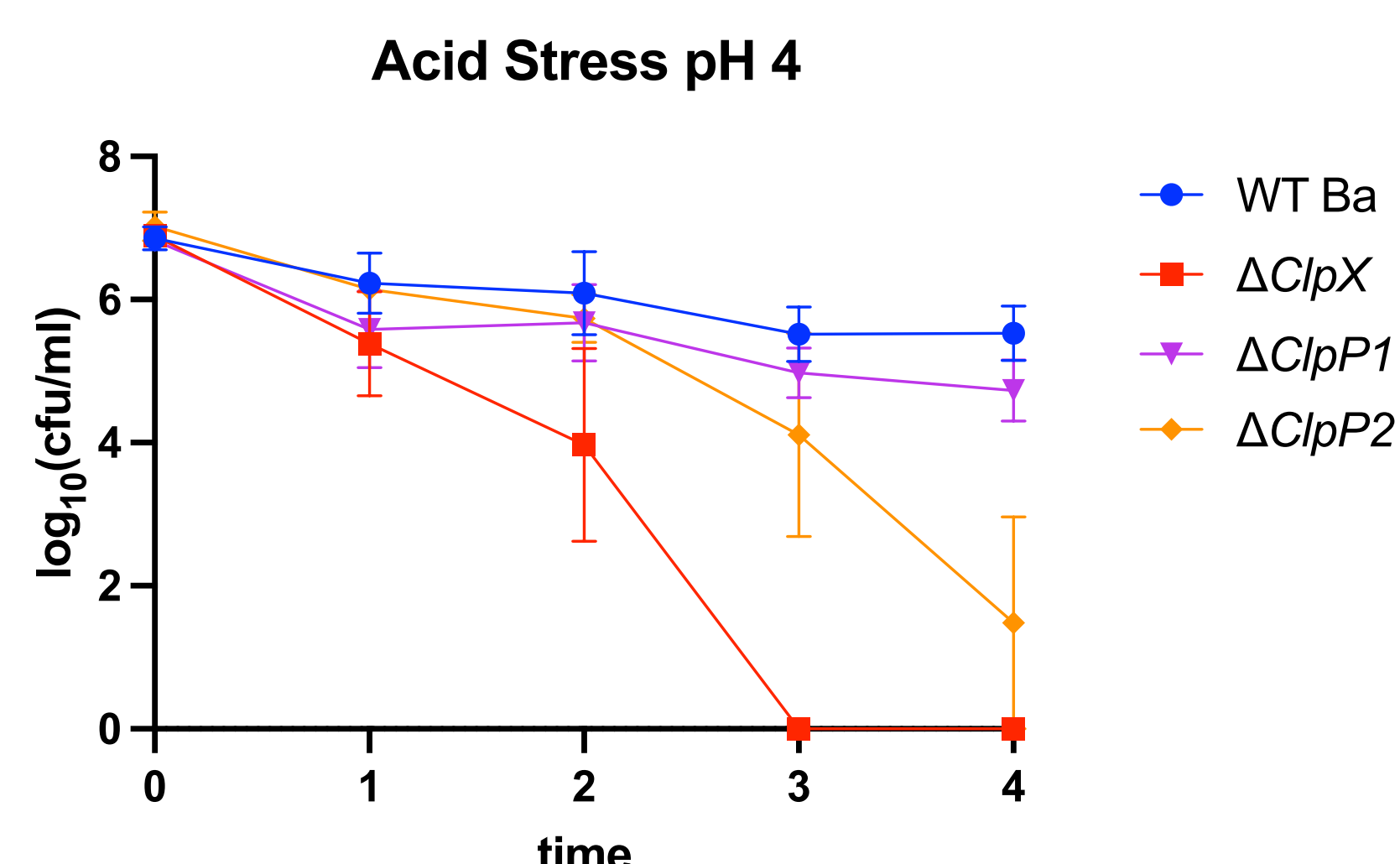


$\Delta ClpX+pClpX^{1264E}$  mutant strain resembles that of attenuated strain,  $\Delta ClpX$ , suggesting chaperone activity is not dependent mechanism of action, and illustrating protease formation to be important for virulence *in vivo*.

## ClpXP formation is necessary for resistance against acid stress

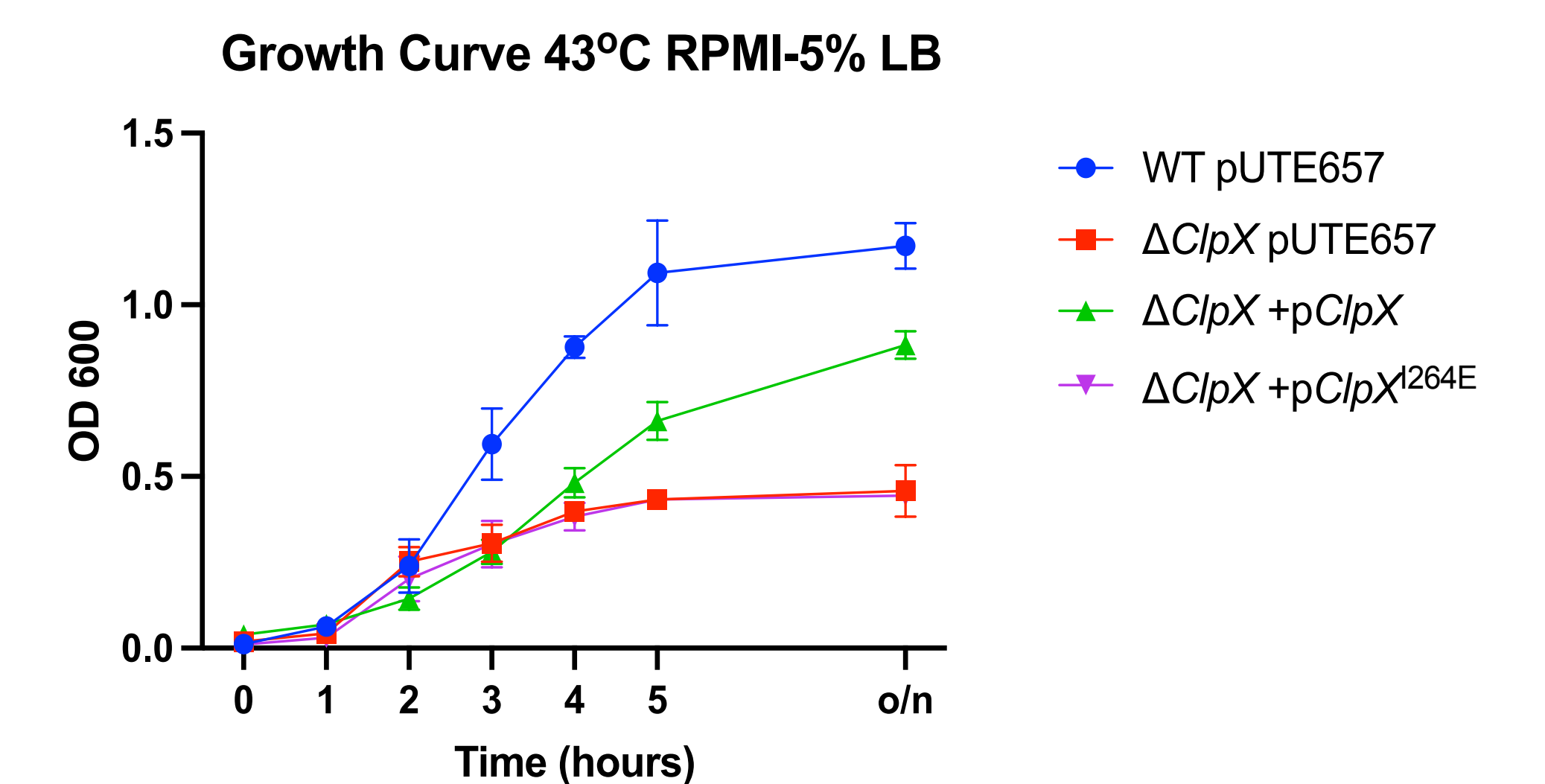


$\Delta ClpX+pClpX^{1264E}$  mutant strain resembles that of attenuated strain,  $\Delta ClpX$ , suggesting chaperone activity is not dependent mechanism of action, and illustrating protease formation to be important in response to acid stress.

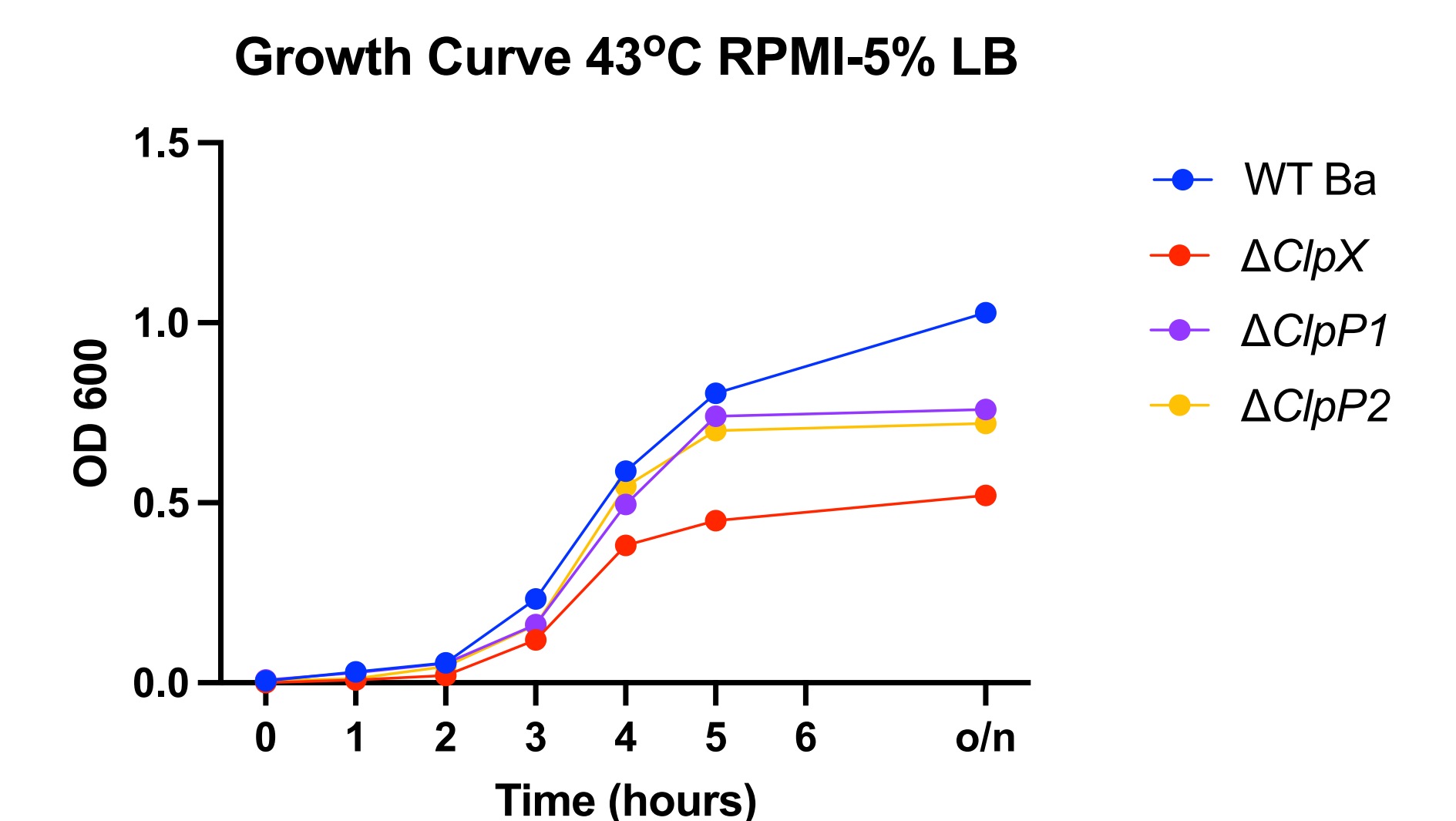


Acid stress test done on the individual components of the ClpXP system suggest that  $\Delta ClpP1$  and  $\Delta ClpP2$  could be compensating for each other. Moreover, data suggests that the  $\Delta ClpP2$  peptidase subunit could hold a more important role than the  $\Delta ClpP1$  subunit.

## ClpXP formation is necessary for resistance against heat stress



$\Delta ClpX+pClpX^{1264E}$  mutant strain resembles that of attenuated strain,  $\Delta ClpX$ , suggesting chaperone activity is not dependent mechanism of action, and illustrating protease formation in response to heat stress.



Heat stress test done on the individual components of the ClpXP system suggest that  $\Delta ClpP1$  and  $\Delta ClpP2$  could be compensating for each other resulting in the intermediate phenotype shown at 43°C.

## Future Projections

- Create the double knock out of *ClpP1* & *ClpP2*
- Repeat *in vivo* survival assay in the *G. mellonella* infection model with double knockout to confirm protease dependent activity.
- Repeat acid and heat stress response assays to confirm protease dependent activity with new double knockout.

## Acknowledgements

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