Biological roles of Type VII substrate proteins in Staphylococcus aureus

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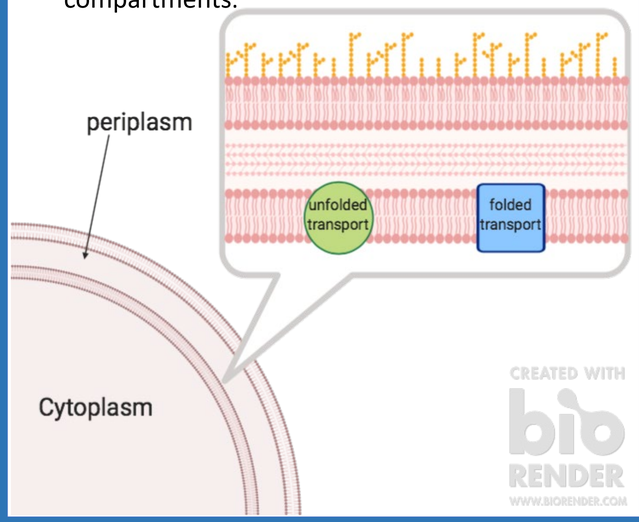
Introduction

The Bacterium, Staphylococcus aureus, more commonly known as MRSA, colonises mammals and is frequently associated with skin infections. To colonise a host and cause disease, S. aureus must compete with the residing microbes. This may be achieved via the secretion of toxins which target other microorganisms. S. aureus contains a Type VII Secretion System(T7SS) which has been shown to secrete 2 toxic substrate proteins to achieve such goals[(1)]. The Palmer Lab, however, has indicated that further T7SS-dependant proteins mediating pathogenicity must exist[(1)]. The genes; EsxB, EsxC, and EsxD, each encode a protein secreted by the T7SS, however, their functions are currently unknown.

Aims:

- To determine whether esxB, esxC, or esxD are toxic to E. coli when produced in 2 different compartments: the cytoplasmic and periplasmic compartments.



Methods

- DNA from each gene was isolated and inserted into a circular piece of DNA with and without coding sequencing for each method of periplasm transport.
- DNA then inserted into and E.coli vector and grown on agar plates.
- Toxicity assay was performed in triplicate for each culture of E.coli.

Results

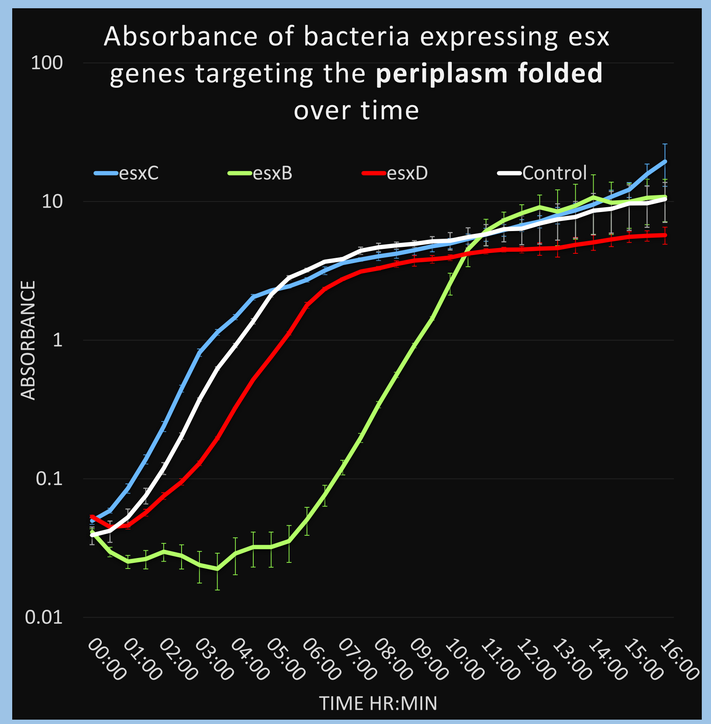


Figure 1.

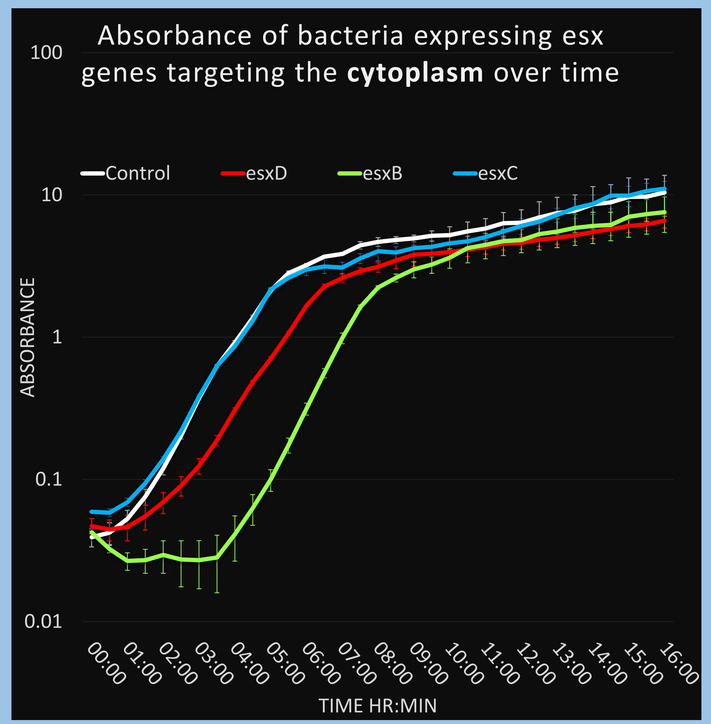


Figure 2.

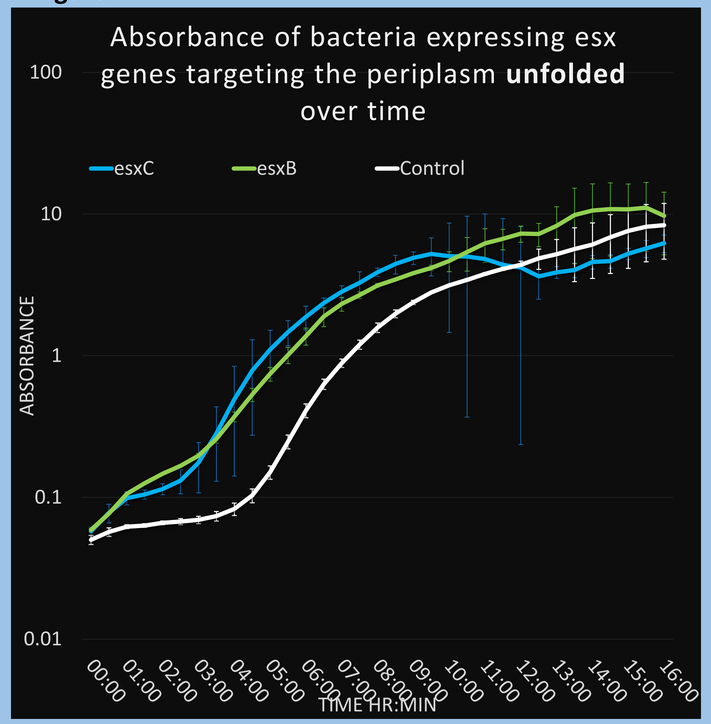


Figure 3. esxD could not be expressed in the periplasm through unfolded transport

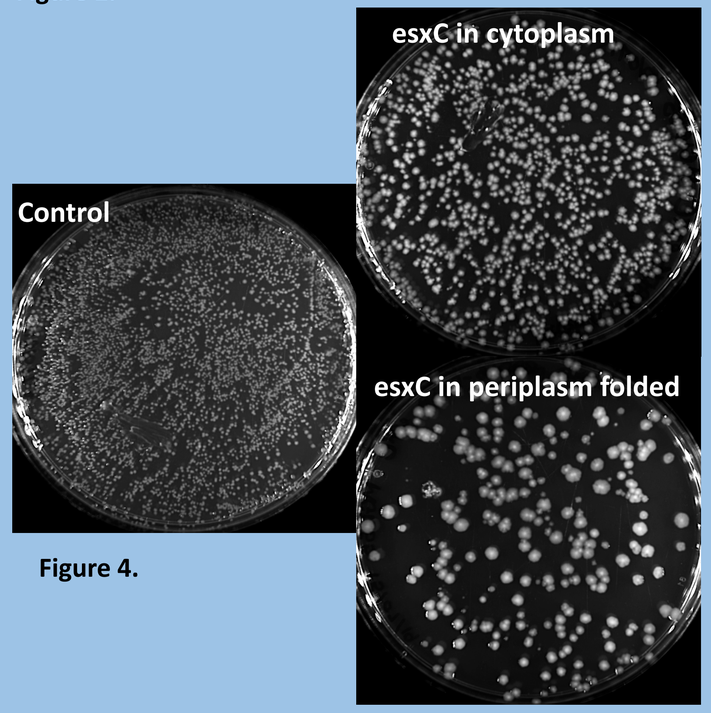


Figure 4.

Conclusions

- Inability to transport esxD to the periplasm unfolded without killing suggests **toxicity.**
- esxB causes a lag in bacterial cell growth growth early on when expressed folded in the periplasm and in the cytoplasm, though more so in the former.
- esxC showed increased colony sizes when in the cytoplasm and when transported to the periplasm folded compared to the control. Microscopy revealed no difference in cell size.

Outstanding questions

- How does esxD mediate cell killing when transported to the periplasm unfolded?
- What mechanism does esxB us to slow growth?
- Is the recovery of esxB due to an unintentional mutation?
- Is the larger colony sizes of esxC due to increased secretion of proteins and/or sugars?

Acknowledgements

Many thanks to Professor Tracy Palmer and Dr Lisa Bowman for their support and expertise. I would also like to thank the Society for Applied Microbiology for their funding of this project.

References

1. Cao, Z., Casabona, M., Kneuper, H., Chalmers, J. and Palmer, T. (2016). The type VII secretion system of Staphylococcus aureus secretes a nuclease toxin that targets competitor bacteria. *Nature Microbiology*, 2(1).