A *Clostridioides difficile* endolysin modulates toxin secretion without cell lysis

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INTRODUCTION

Protein secretion is essential for prokaryotic life, ensuring bacterial survival, proliferation, and dissemination

Two main Gram positive bacterial secretion systems (Sec and Tat) facilitate transport of proteins that have specific signal sequences[1]

***Clostridioides difficile,* the primary causative agent of antibiotic associated diarrhea, produces two large glucosylating toxins, TcdA and TcdB, which lack known signal sequences[2,3], leaving their secretion mechanism unclear**

However, a novel holin-like protein was clearly shown to be required for toxin secretion[2,3]. Holins, typically associated with bacteriophages, form part of holin-endolysin (lysin) systems

Holins form pores in cell membranes, allowing lysins to reach and cleave the peptidoglycan (PG), enabling cellular content release

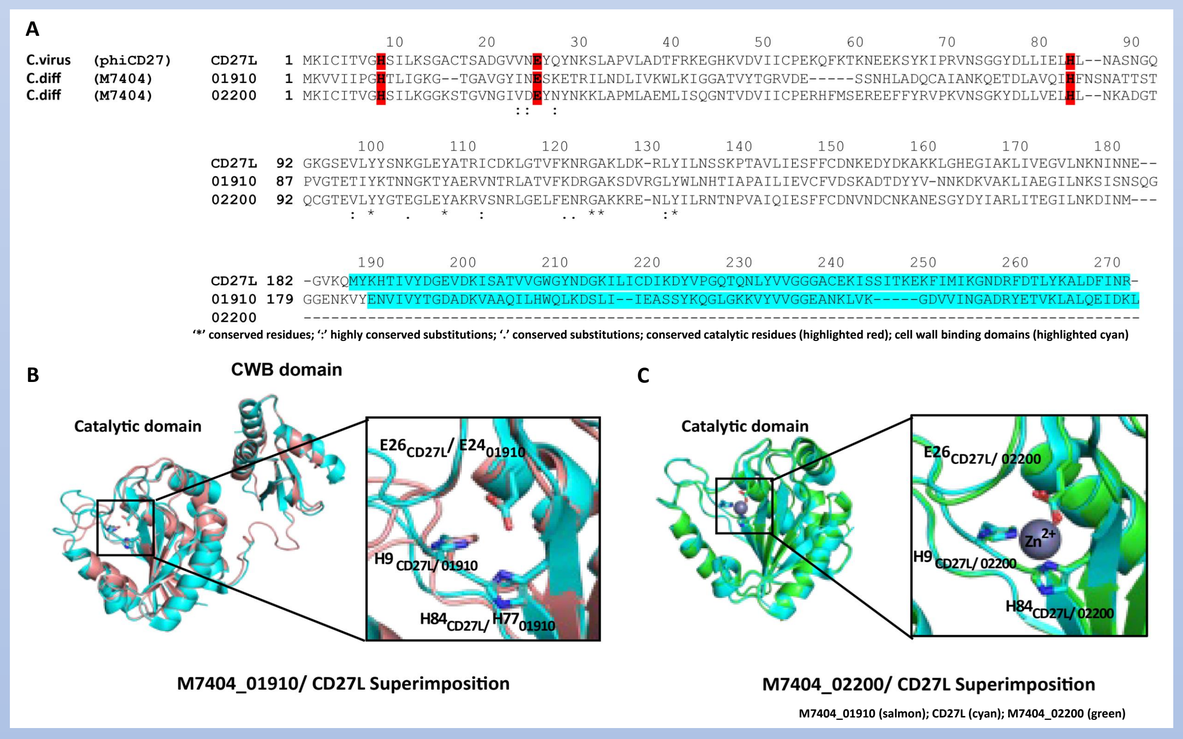
AIM

To determine if two highly conserved putative *C. difficile* lysins; M7404_01910 and M7404_02200, are involved in the extracellular release of TcdA and TcdB

RESULTS

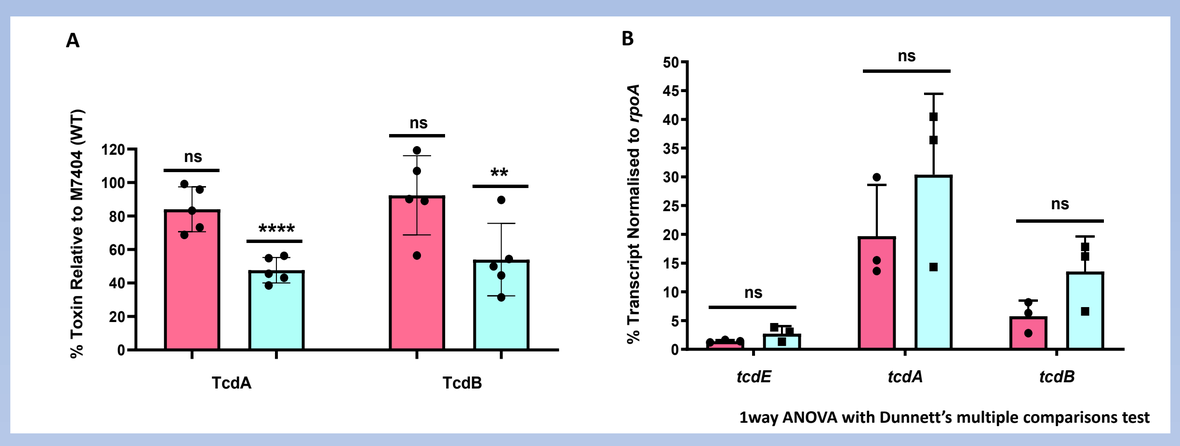
M7404_01910 & M7404_02200 are likely to encode for catalytically active lysins

M7404_01910 & M7404_02200 share significant similarity in amino acid sequence (A) & 3D structure (Alphafold) with the functional *C. difficile* phage-encoded lysin CD27L (B,C)



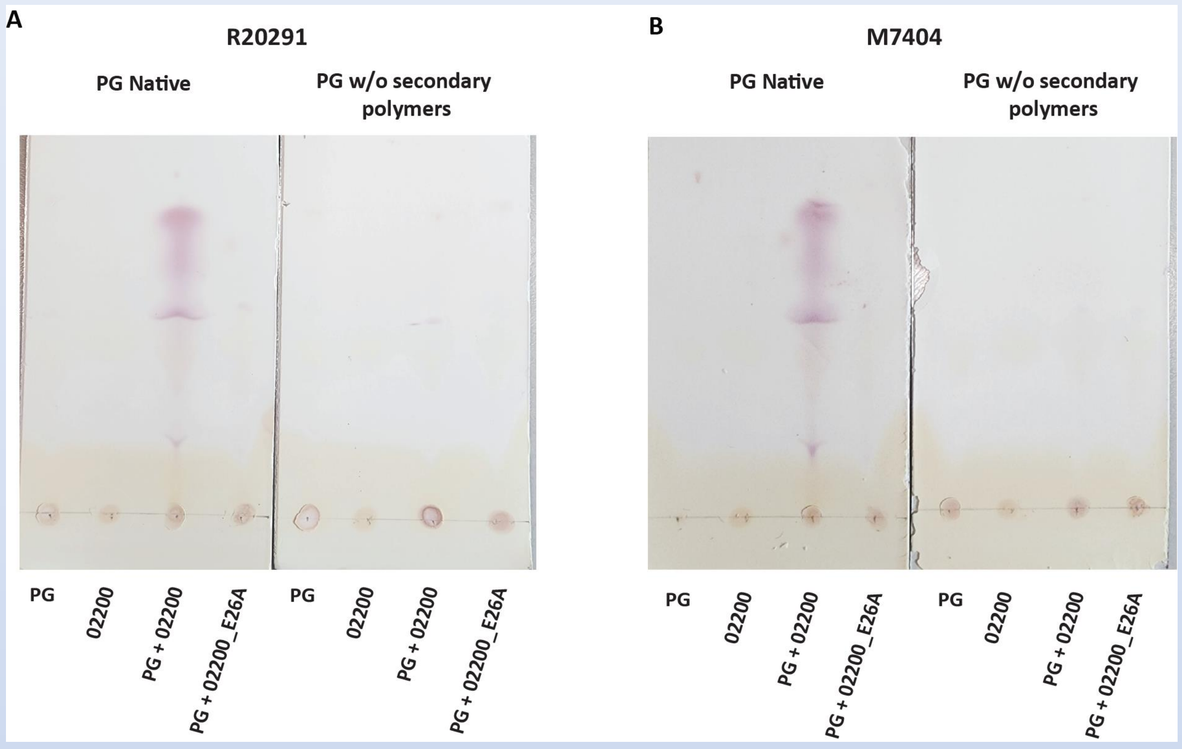
M7404_02200 mutant shows reduced TcdA & TcdB release

TcdA & TcdB release only significantly reduced in the *M7404_02200* mutant (teal) compared to wild type (pink) (A); secretion deficit not due to differences in *tcdE*, *tcdA* or *tcdB* transcription (B)



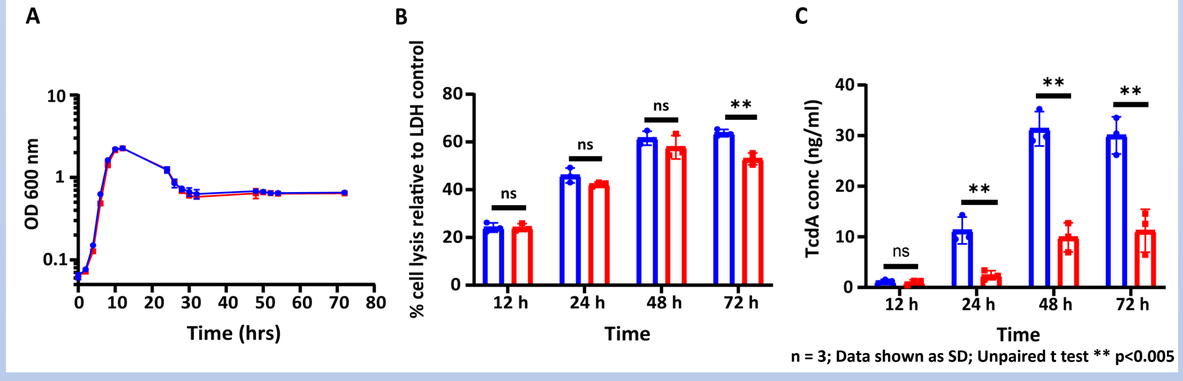
M7404_02200 functions as a lysin *in vitro*

Clear hydrolysis observed for native R20291 & M7404 PG samples treated with M7404_02200 (PG + 02200) (A,B). No hydrolysis observed in all negative controls (PG or 02200 alone, with catalytic mutant (PG + 02200_E26A) or without secondary polymers)



Toxin release by M7404_02200 occurs in a non-lytic manner

M7404_02200 mutant (red) shows no difference in growth* (A) or cell lysis (LDH release) (B) compared to WT (blue) but shows a toxin secretion deficit across time (C)



CONCLUSIONS

Bioinformatic analysis identified two putative lysins, M7404_01910 & M7404_02200 in *C. difficile* M7404, with similarity to the *C. difficile* phage-encoded lysin, CD27L

Only mutation in the gene encoding M7404_02200 affects toxin release (~ 50 % reduction in TcdA & TcdB release)

Reduction in toxin release for the *M7404_02200* mutant is not due to alteration in toxin gene or holin gene transcription

Purified recombinant M7404_02200 was shown to be a functional lysin *in vitro,* able to hydrolyse PG from R20291 & M7404

M7404_02200 modulates toxin secretion *via* a non-lytic mechanism

REFERENCES

[1] Natale, P., Bruser, T. & Driessen, A. J. Sec- and Tat-mediated protein secretion across the bacterial cytoplasmic membrane–distinct translocases and mechanisms. Biochim Biophys. Acta 1778, 1735–1756 (2008)

[2] Govind, R. & Dupuy, B. Secretion of Clostridium difficile toxins A and B requires the holin-like protein TcdE. PLoS Pathog. 8, e1002727 (2012)

[3] DiBenedetto *et al*. *Clostridioides difficile*'s virulence requires efficient holin-mediated toxin secretion. iScience 28, 112586 (2025)

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don whitley scientific culturing innovation

* Whitley Workstation required for *C. difficile* growth

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