Defining the Molecular Basis of *Clostridioides difficile* Adaptation to Reactive Nitrogen Species

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Introduction

- *Clostridioides difficile* (*C. difficile*) is a Gram-positive, spore forming, toxin- producing anaerobic bacteria. It is the leading cause of hospital-acquired diarrhea, leading to colon inflammation (colitis)[1,2].
- During *C. difficile* infection (CDI), the host innate immune cells produce bactericidal molecules including reactive nitrogen species (RNS), such as nitric oxide (NO) and Peroxynitrite ($ONOO^-$)[3,4].
- Reactive Nitrogen and Oxygen species (RNS/ROS)-based therapies are emerging alternate antimicrobial strategies as they can inhibit bacterial growth.

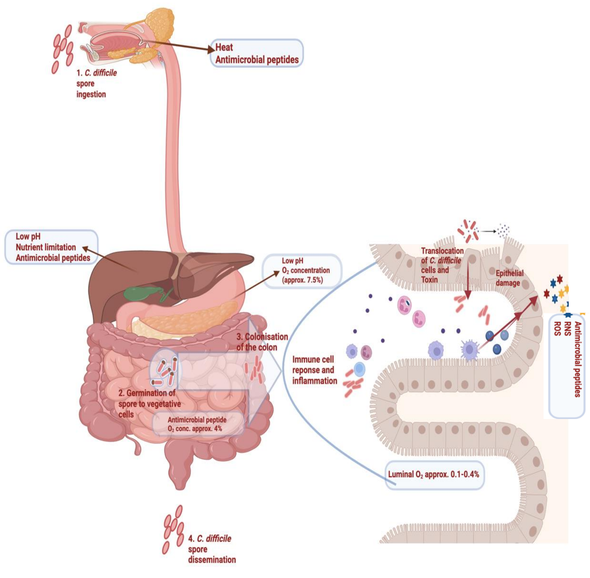

Fig. 1. Host- derived antimicrobial defenses targeting *C. difficile* in the gut

Objective

Given *C. difficile* demonstrated ability to adapt and persist in the gut despite host-generated RNS, the bacterium has likely evolved specialized mechanisms to resist RNS stress. We aim to investigate the molecular basis of RNS resistance in *C. difficile* and its implications for virulence

Methods

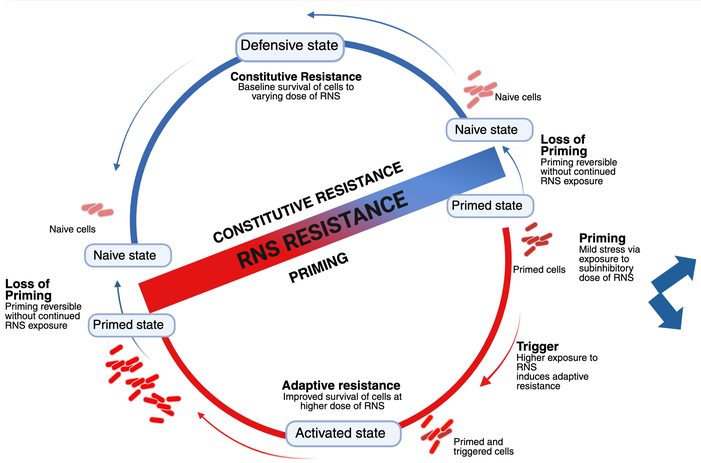

Fig. 2. Principle of constitutive and adaptive (priming) RNS resistance

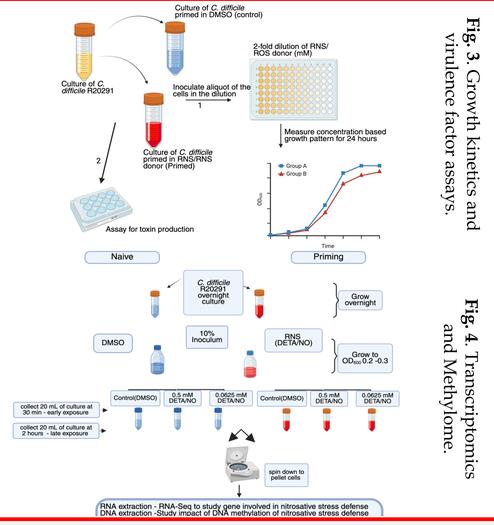

Fig. 3. Growth kinetics and virulence factor assays.

Fig. 4. Transcriptomics and Methylome.

Conclusions

- RNS concentration-dependent growth of *C. difficile* reflects its innate resistance to RNS. Priming further enhances survival under elevated nitrosative stress. These findings indicate that *C. difficile* uses both constitutive (innate) and adaptive (priming) strategies to resist RNS[5-7].
- Unlike its response to RNS, priming with RNS does not enhance *C. difficile* resistance to elevated ROS; in fact, it may even be disadvantageous .
- RNS-exposed *C. difficile* produces less toxin, suggesting a cellular preference for allocating resources toward resistance rather than virulence.

Results

I. *C. difficile* tolerates low levels of RNS/ROS

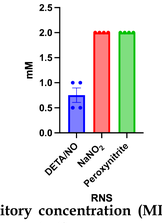

Fig. 5. Minimum inhibitory concentration (MIC) of *C. difficile*. *C. difficile* tolerates different RNS donors at varying concentrations

II. Priming increases resistance to RNS in *C. difficile*

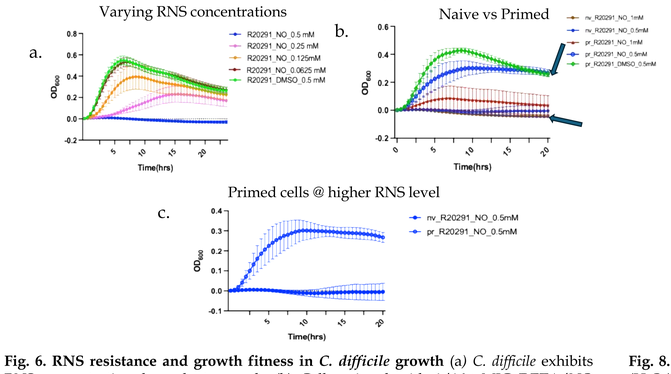

Fig. 6. RNS resistance and growth fitness in *C. difficile* growth (a) *C. difficile* exhibits RNS concentration-dependent growth. (b) Cells primed with 1/16× MIC DETA/NO (NO) tolerate otherwise deleterious concentrations. (c) Primed *C. difficile* displays enhanced growth fitness at elevated DETA/NO (0.5 mM), where naive cells failed to grow.

III. Priming does not increase resistance to ROS in *C. difficile*

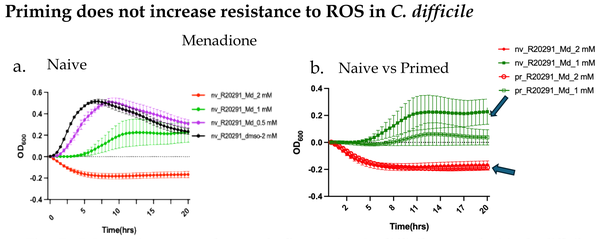

Fig. 7. ROS resistance and growth fitness in *C. difficile* growth (a) *C. difficile* exhibits ROS (Menadione) concentration-dependent growth (b) Menadione primed cells at 1/16X MIC (pr) do not have increased fitness against Menadione.

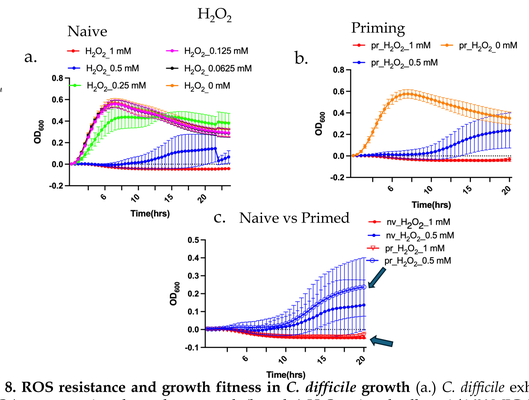

Fig. 8. ROS resistance and growth fitness in *C. difficile* growth (a.) *C. difficile* exhibits ROS (H_2O_2) concentration-dependent growth (b and c) H_2O_2 primed cells at 1/16X MIC (pr) do not exhibit increased fitness against H_2O_2.

IV. RNS exposure reduces toxin (TcdA/B) production in *C. difficile*

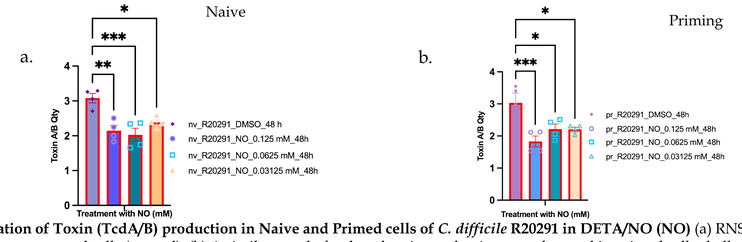

Fig. 9. Quantification of Toxin (TcdA/B) production in Naive and Primed cells of *C. difficile* R20291 in DETA/NO (NO) (a) RNS exposed cells produce lower toxin levels compared to unexposed cells (control). (b) A similar trend of reduced toxin production was observed in primed cells challenged with RNS, compared to primed, non-RNS-challenged control cells.

Future Directions

Future studies will use multi-omics approaches, including RNA-seq, Tn-seq and methylome analysis, to elucidate the genome-wide molecular mechanism underlying both constitutive and priming based RNS resistance in *C. difficile*.

References

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Acknowledgements

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