Clostridioides difficile exploits toxin-mediated inflammation to alter the host nutritional landscape and exclude competitors from the gut microbiota

NC STATE UNIVERSITY College of Veterinary Medicine

cmpike@ncsu.edu

Fletcher, J.R.[1], **Pike, C.M.**[1], Parsons, R.J.[1] Rivera, A.J.[1], Foley, M.H. [1], Montgomery S.A[2], Theriot, C.M.[1]

[1]Department of Population Health and Pathobiology, North Carolina State University, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

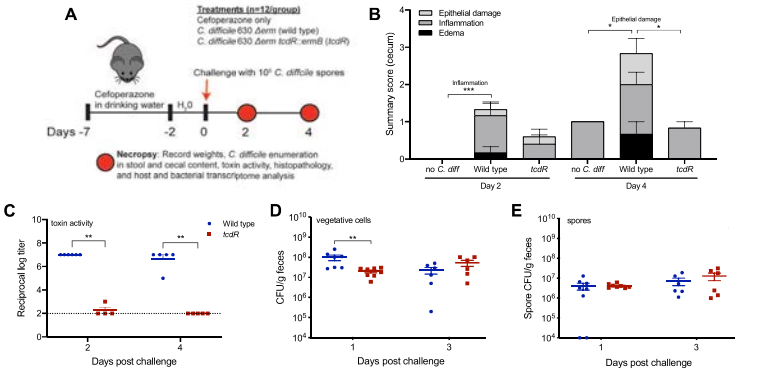
[2]Department of Pathology and Laboratory Medicine, Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC

Introduction

Clostridioides difficile infection (CDI) is a significant public health problem associated with increasing morbidity, mortality, and health-care related costs around the globe. CDI is characterized by a robust immune response caused by two large toxins, resulting in extensive tissue damage by weakening the mucosal barrier and exacerbating inflammation. Toxins are expressed under nutrient-limiting conditions, leading us to hypothesize that toxins induce inflammation to gain access to a different pool of nutrients. Using a toxin-deficient mutant strain of *C. difficile* in a mouse model, we used an omics approach to define how toxin-induced inflammation alters *C. difficile* metabolism, tissue gene expression, and the gut microbiota to determine if host inflammation provides an alternative niche for *C. difficile*.

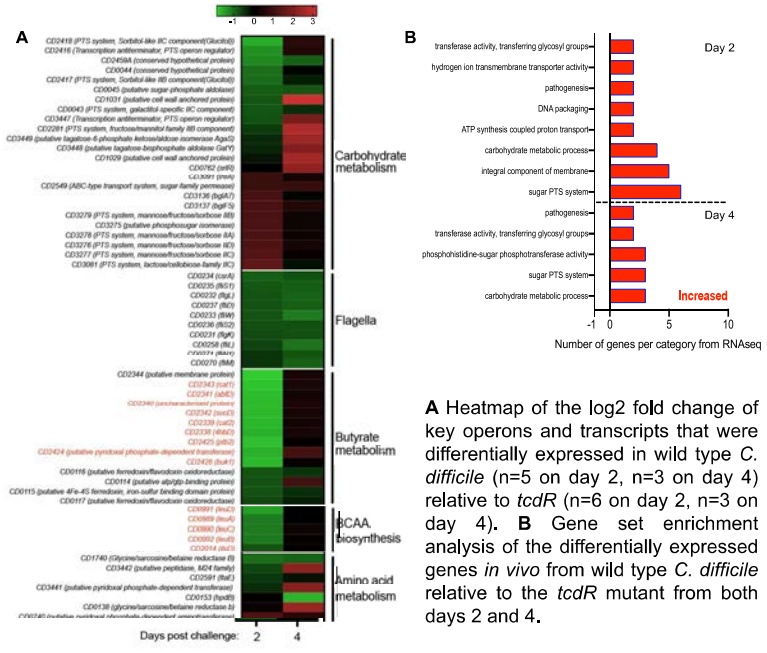
Results

Inflammation is attenuated in *tcdR* mice in a mouse model of *C. difficile* infection



A Schematic depicting experimental design. All mice (n=36) received the antibiotic cefoperazone in their drinking water. Subsets were orally gavaged with wild type (n=12) or *tcdR* (n=12) after antibiotic treatment.). **B** Histopathological summary scores of the cecum. **C** *C. difficile* vegetative cell CFUs in feces (n=6-8 per strain). **D** *C. difficile* spore CFUs in the feces (n=6-8 per strain). **E** Toxin activity in the cecal content of mice (n=4-6).

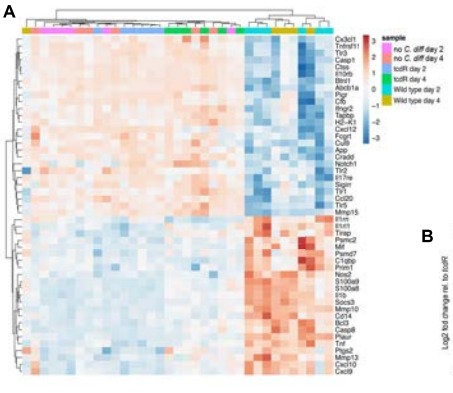
Metabolic gene expression in *C. difficile* is significantly altered by toxin-mediated inflammation



A Heatmap of the log2 fold change of key operons and transcripts that were differentially expressed in wild type *C. difficile* (n=5 on day 2, n=3 on day 4) relative to *tcdR* (n=6 on day 2, n=3 on day 4). **B** Gene set enrichment analysis of the differentially expressed genes *in vivo* from wild type *C. difficile* relative to the *tcdR* mutant from both days 2 and 4.

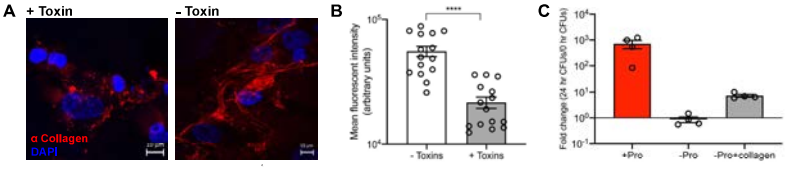
Results

C. difficile induces expression of numerous transcripts associated with inflammation and ECM degradation



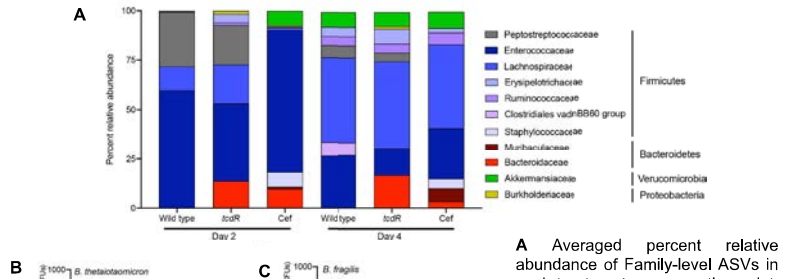
A Heatmap of the top 50 differentially regulated transcripts (by adj. p value) in the ceca of uninfected controls, wild type mice, and *tcdR* mice (n=5-6 per treatment and time point). **B** Log2 fold changes of various *Mmp*s and associated transcripts from wild type vs. *tcdR* mouse ceca.

Toxin-mediated degradation of collagen supports *C. difficile* growth *in vitro*



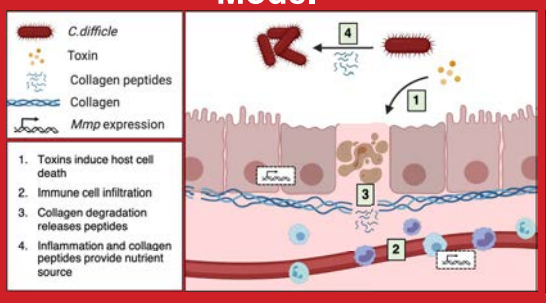
A Collagen (red) produced by IMR90 cells and DAPI (blue); scale bar, 10 μm. **B** Mean fluorescent intensity of collagen produced by IMR90 cells cultured in the presence or absence of toxins calculated using ImageJ. "***" denotes $p < 0.001$ by by Mann-Whitney rank-sum test. **C** *C. difficile* grown in complete CDMM, CDMM lacking proline, or CDMM lacking proline and supplemented with heat-degraded collagen.

C. difficile toxin activity suppresses the Bacteroidaceae that are able to compete with *C. difficile* for amino acids.



A Averaged percent relative abundance of Family-level ASVs in each treatment group per timepoint. ASVs with less than 1% relative abundance in all samples were not included. **B**, **C** 16 hour fold change in CFUs of *B. thetaiotaomicron* and *B. fragilis* in minimal media with or without glucose, supplemented with either proline or hydroxyproline

Model



Funding

NIH NIGMS R35 GM119438
NIH NIGMS K01 GM109236
UNC CGIBD T32DK07737
NCSU CVM internal pilot