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An introduction to virulence factors of bacterial pathogens

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Pathogen and virulence factors

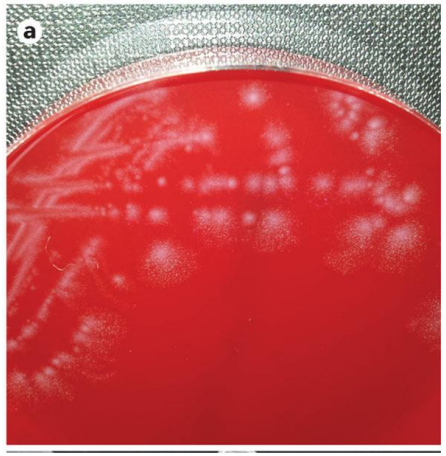
- Pathogen
  - A microbe that disease through infection
  - Can be bacterial, viral, fungal, etc

- Virulence factors (VFs)
  - The molecules (gene products) that allows a microbe to be a pathogen
  - Assist the bacterium colonize the host at the cellular level.
  - Conventional VFs include secreted proteins, such as toxins and enzymes, and cell-surface structures
  - Also include genes that are indirectly involved in pathogenesis, such as secretion machineries, catalases, etc.

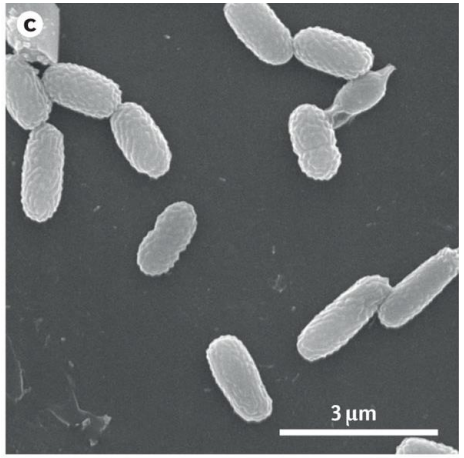
Chen, L., et al. *Nucleic Acids Research*. 2005

Use *Clostridium difficile* (艱難梭菌) as an example

- Spore-forming, Gram-positive, anaerobic bacillus
- Gastrointestinal pathogen; disease associated with *C. difficile* infection (CDI) ranges from mild diarrhea to colitis
- Main cause of antibiotic-associated diarrhea
- In US, about 200,000 people are infected annually. In Hong Kong, incidence of *C. difficile* infection in the Prince of Wales Hospital increased approximately threefold from 2009 to 2013



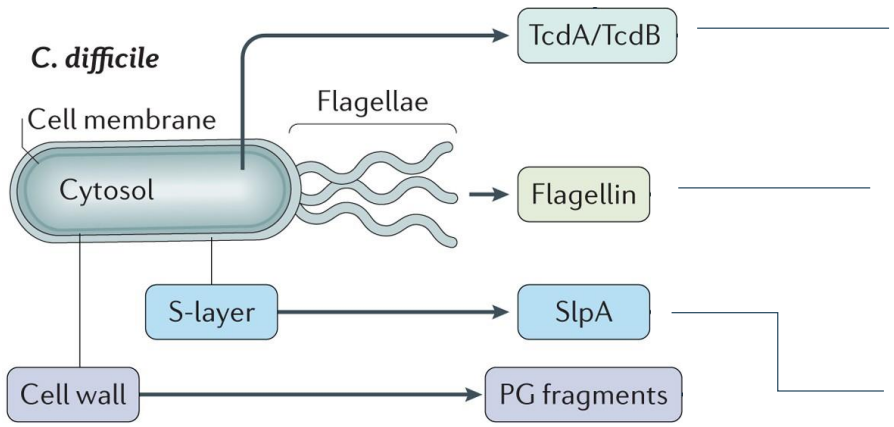
C. difficile colonies on a blood agar plate



Scanning electron micrograph of *C. difficile* spores

Smits, W.K., et al. *Nat Rev Dis Primers*. 2016

Different types of VFs of *C. difficile*



Toxicity

- Large clostridial toxins
- Directly target host cells

Motility

- Promote adherence to host cells
- Facilitate translocation of virulence factors across cell membranes

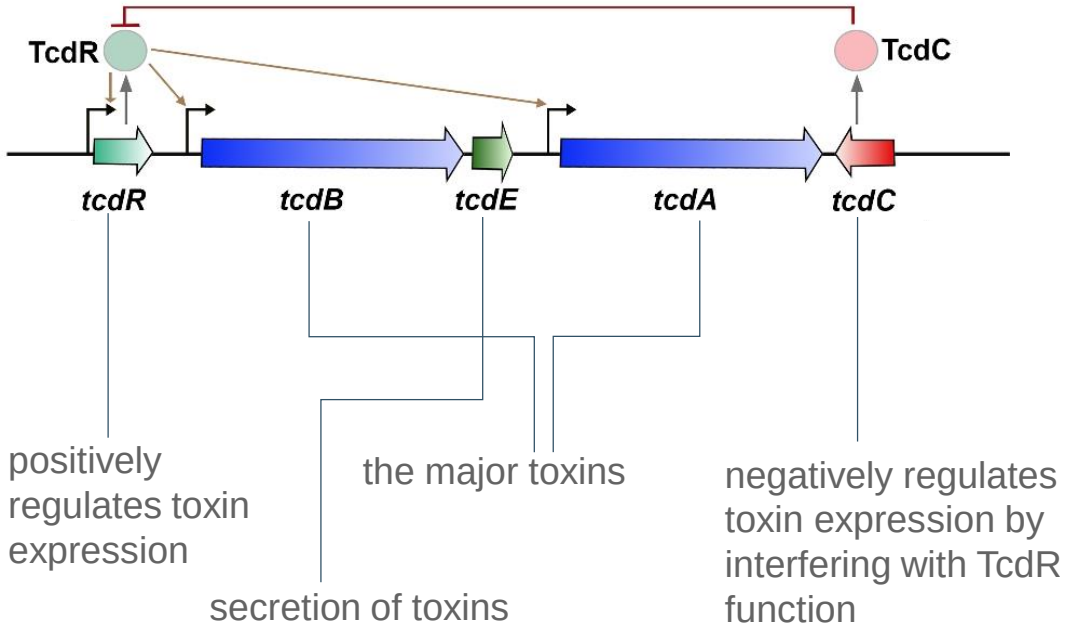
Adherence

- Bound to the cell wall
- Formed of SlpA and decorated with cell wall protein (CWP) family
- Promote aggregation
- a highly deacetylated peptidoglycan (PG) cell wall containing unique secondary cell wall polymers
- maintain cell shape and integrity, and anchor cell wall proteins (CWP)

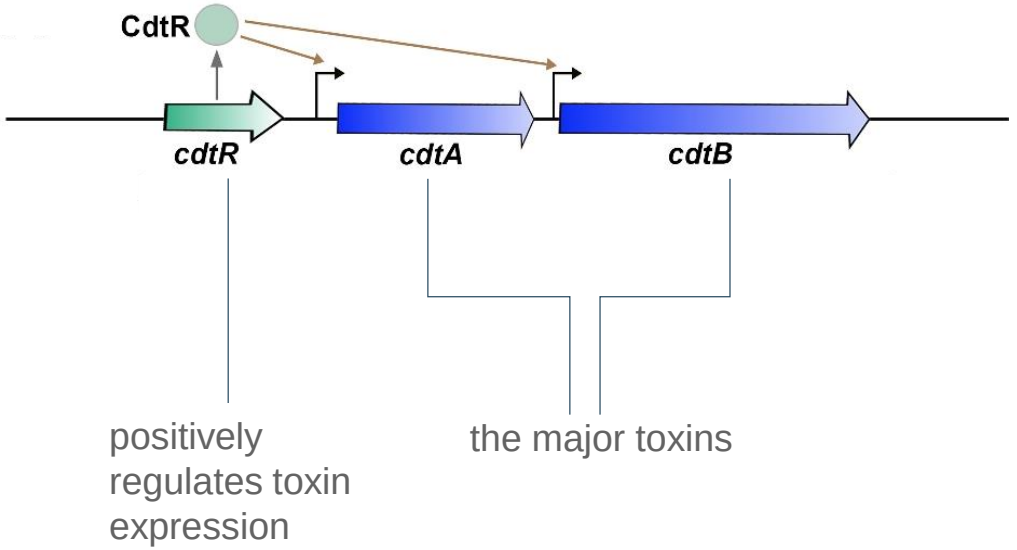
Kirk, J.A. et al. *Microb Biotechnol*. 2016. Rasko, D. et al. *Nat Rev Drug Discov*. 2010

Regulation of the *C. difficile* toxins.

PaLoc (19.6 Kb)

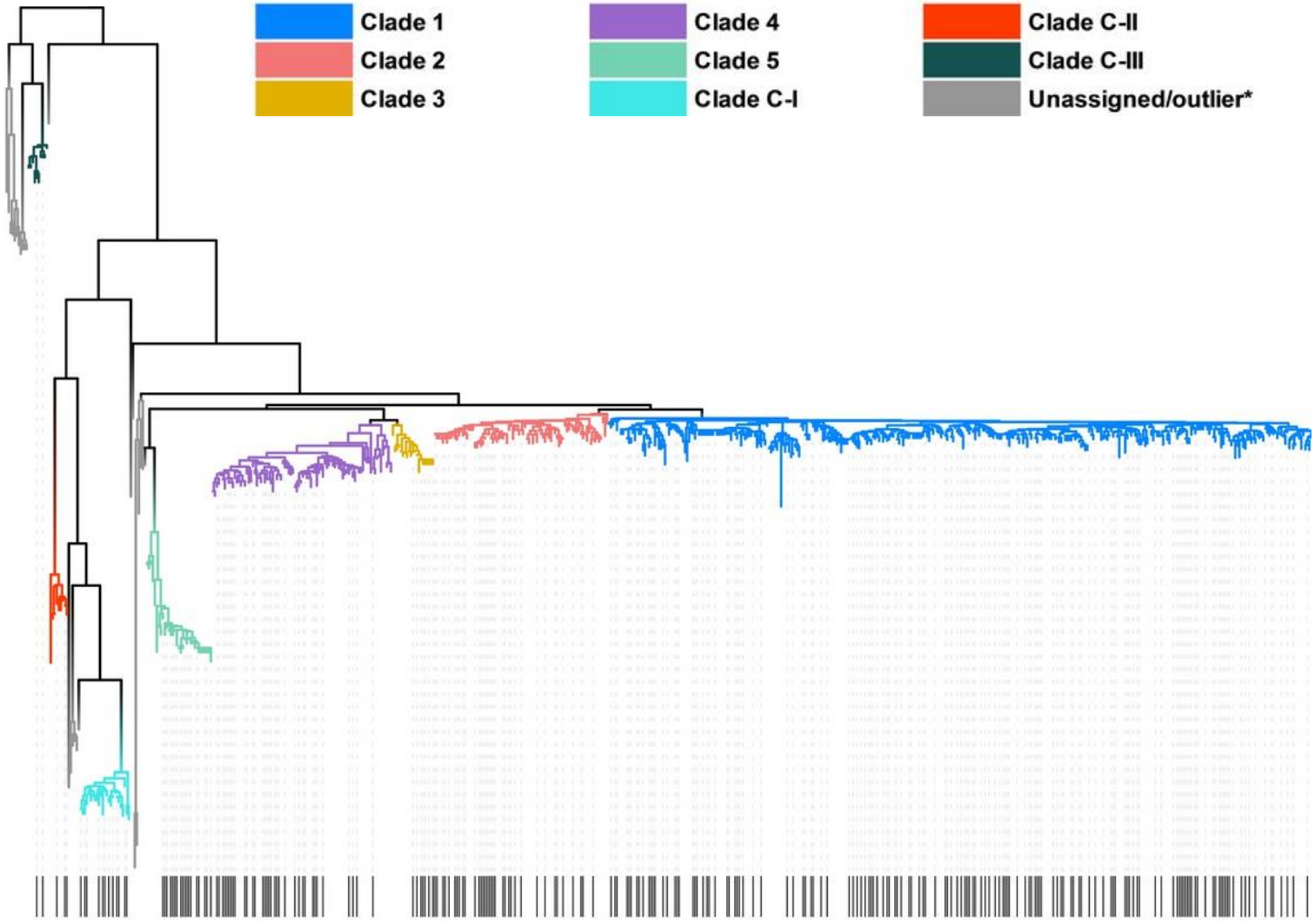


CdtLoc (6.2 Kb)



Chandrasekaran, R. et al. FEMS Microbiology Reviews. 2017

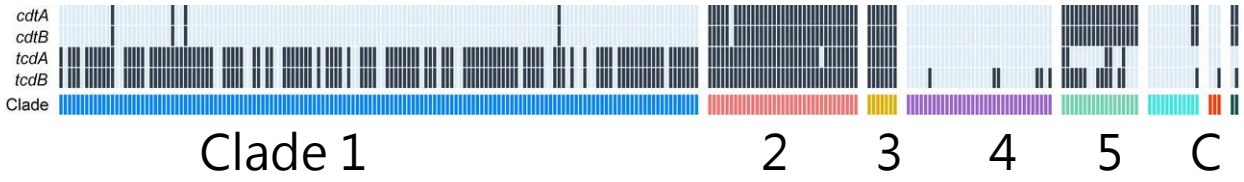
Analysis of 12,621 C. difficile genomes reveals 5 distinct clades



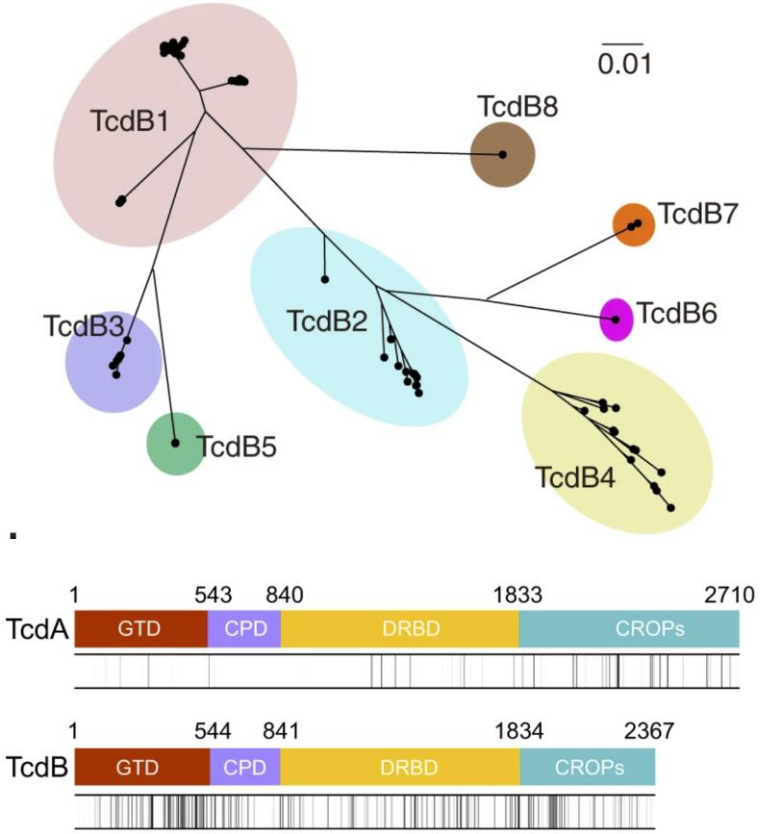
- Phylogeny tree is constructed to show to evolutionary relationships among all the genomes
- Taxonomically divergent clades

Knight, D.R., et al. *Elife*. 2021

Toxins are also divergent across clades



TcdA	TcdB	CDT	Fraction
+	+	-	60.4%
+	+	+	26.2%
-	-	+	4.3%
-	+	-	3.7%



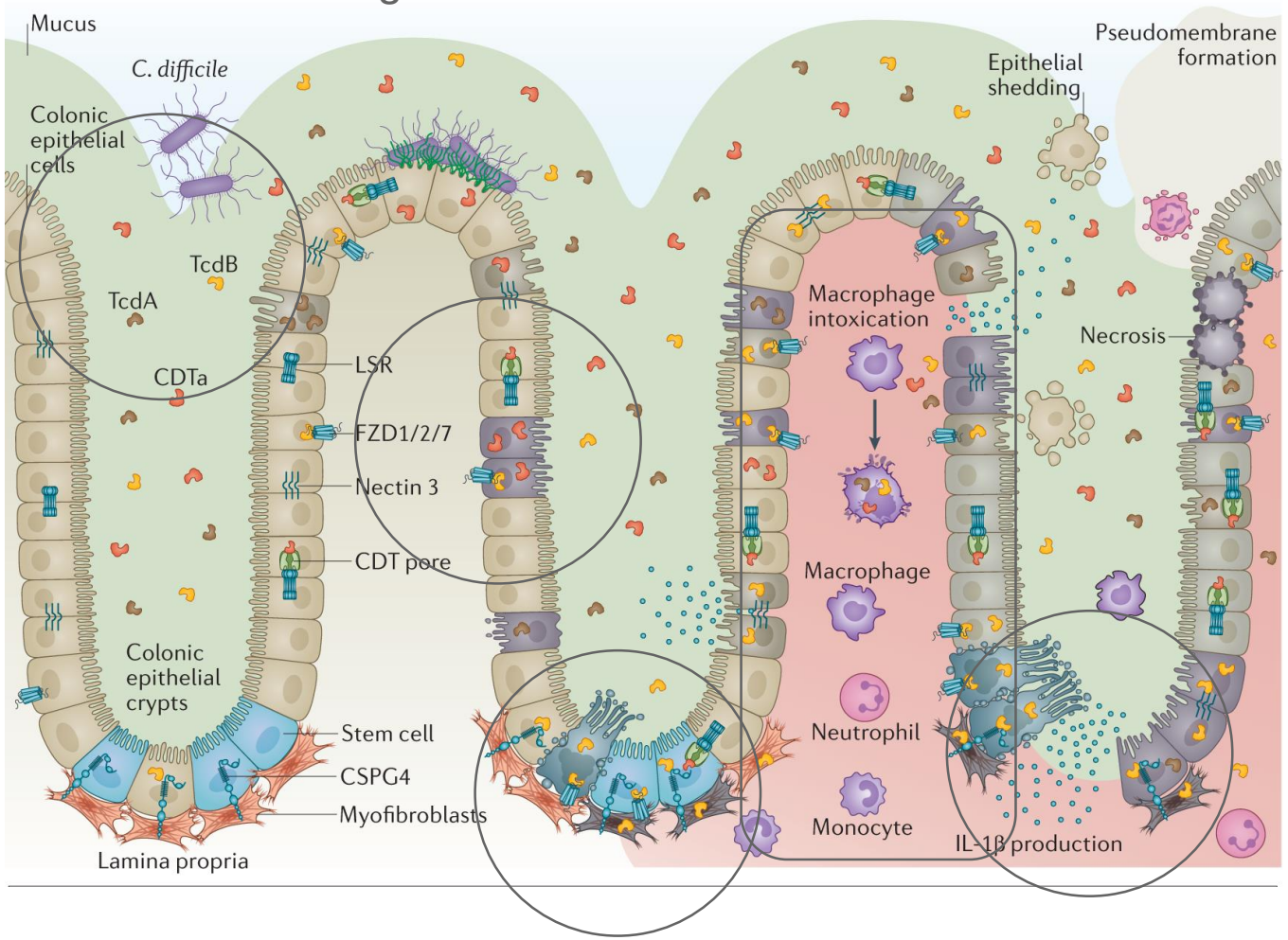
TcdB was much more diverse in amino-acid sequence than TcdA, suggesting a complex and accelerated evolution of the *tcdB* gene

Knight, D.R., et al. *Elife*. 2021

Host response to C. difficile toxins

5. The intoxication of monocytes and macrophages promote IL-1β production, which leads to further inflammation and damage within the tissue

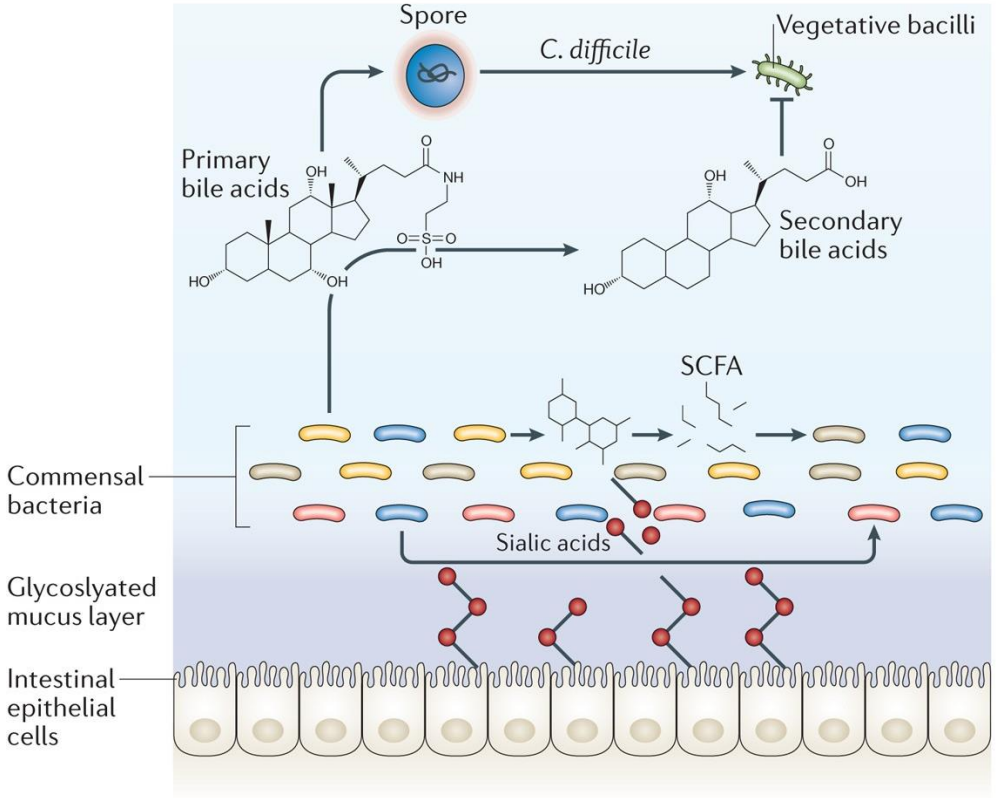
4. Damaged epithelial cells release cytokines, recruit immune cells



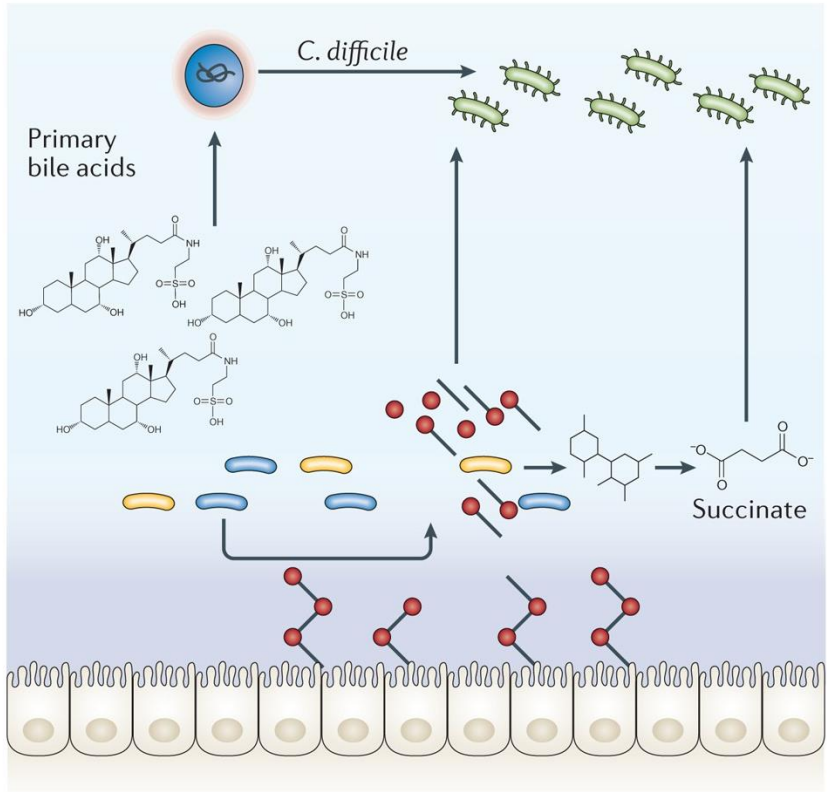
Rasko, D. et al. *Nat Rev Drug Discov*. 2010

Microbiota-mediated defenses against *C. difficile*

The intact microbiota converts primary bile acids into secondary bile acids, which inhibit the growth of *C. difficile*



Antibiotic-mediated disruption of the microbiota depletes primary bile acid converters



Abt, M.C.et al. *Nat Rev Microbiol*. 2016

Use sequencing data to characterize CDI-related changes systematically

Nature Communications. 2021

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

GUT MICROBIOTA

Clostridioides difficile uses amino acids associated with gut microbial dysbiosis in a subset of patients with diarrhea

Eric J. Battaglioli[1]*, Vanessa L. Hale[2,3]*, Jun Chen[4], Patricio Jeraldo[2], Coral Ruiz-Mojica[1], Bradley A. Schmidt[1], Vayu M. Rekdal[1], Lisa M. Till[1], Lutfi Huq[2], Samuel A. Smits[5], William J. Moor[1], Yava Jones-Hall[6], Thomas Smyrk[7], Sahil Khanna[1], Darrell S. Pardi[1], Madhusudan Grover[1], Robin Patel[8], Nicholas Chia[2], Heidi Nelson[2], Justin L. Sonnenburg[5], Gianrico Farrugia[9], Purna C. Kashyap[1,10†]

Host response to *C. diff* infection

Science Translational Medicine. 2018

ARTICLE

Check for updates

https://doi.org/10.1038/s41467-020-20746-4 OPEN

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

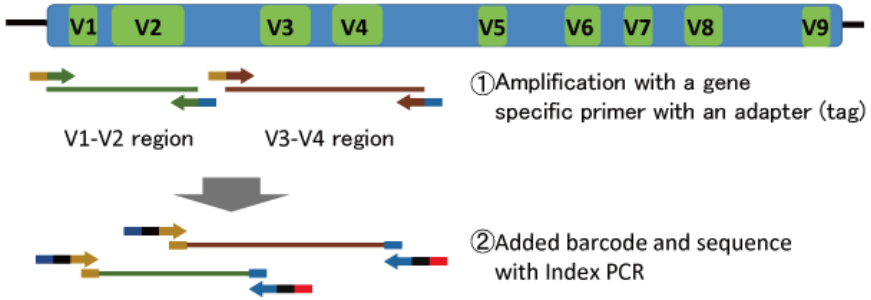
Joshua R. Fletcher[1], Colleen M. Pike[1], Ruth J. Parsons[1], Alissa J. Rivera[1], Matthew H. Foley[1], Michael R. McLaren[1], Stephanie A. Montgomery[2] & Casey M. Theriot[1✉]

C. diff explores disrupted microbial community

Measure microbial and host transcriptional activity using sequencing

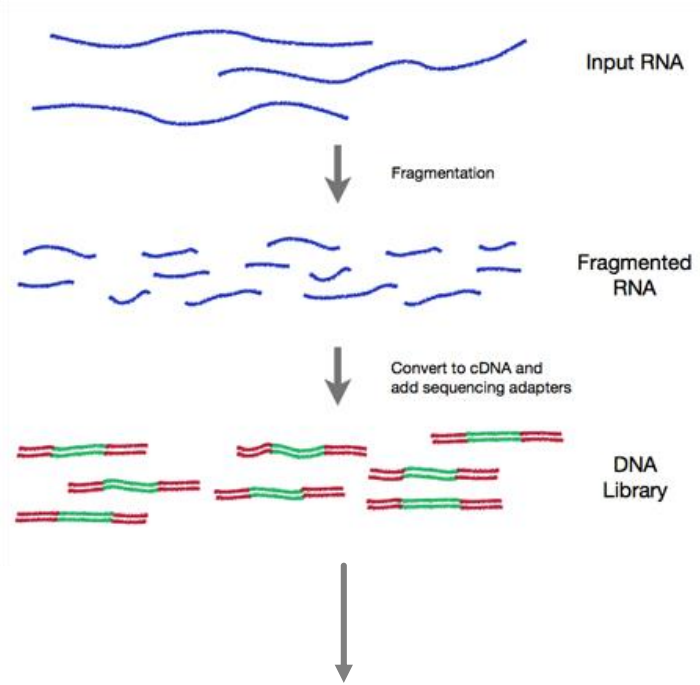
Microbial

16S rRNA genes are conserved among bacterial species
9 High Variable Regions (V1 – V9)



- Abundance of all the bacteria
- Potentially reflect the activity of the microbial community

Microbial Host



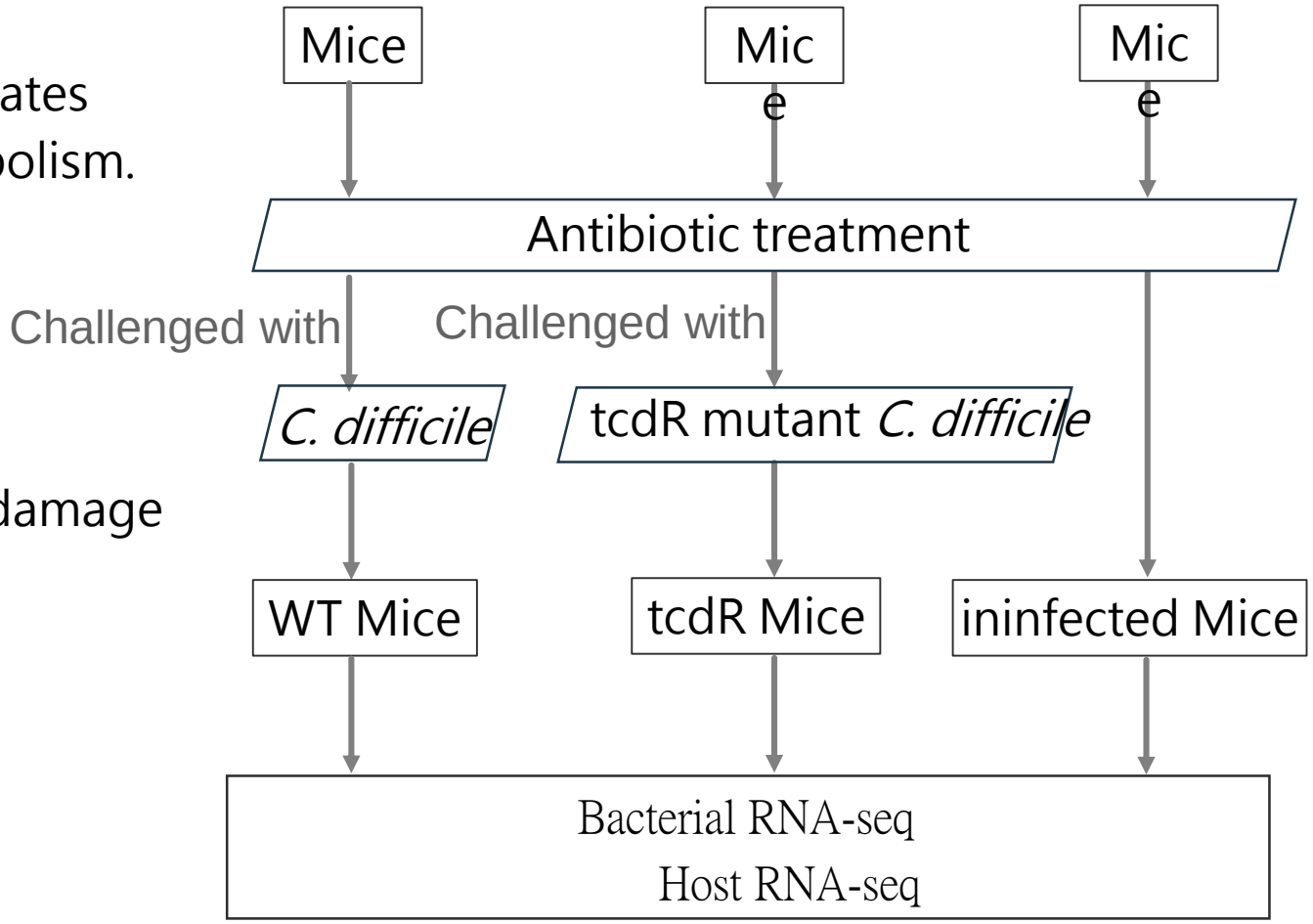
- For host and single bacterial genomes
  - Transcriptional abundance of all the genes in the genom
- For microbial community
  - Transcriptional activities of all the genes in all the genomes

https://www.repertoire.co.jp/en/research/technology/16srrna

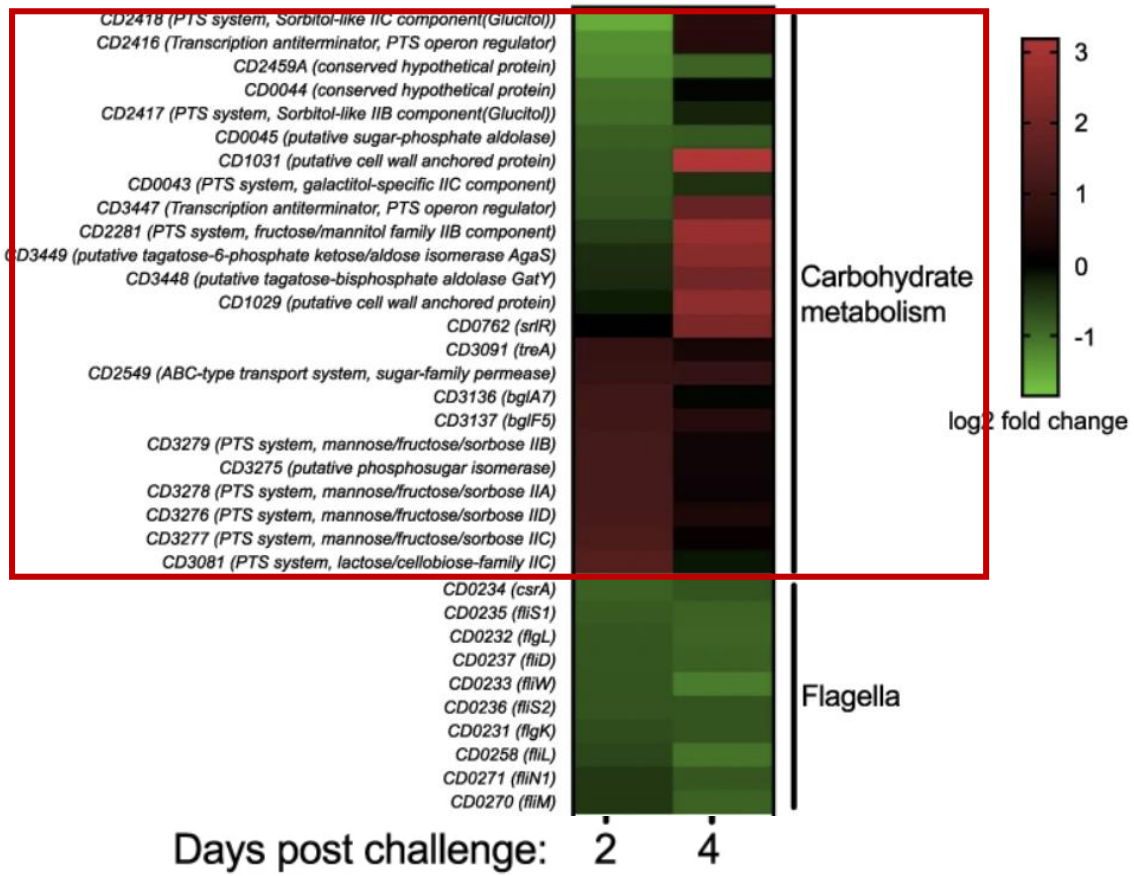
Whether the pathogen can exploit an inflamed environment in order to thrive

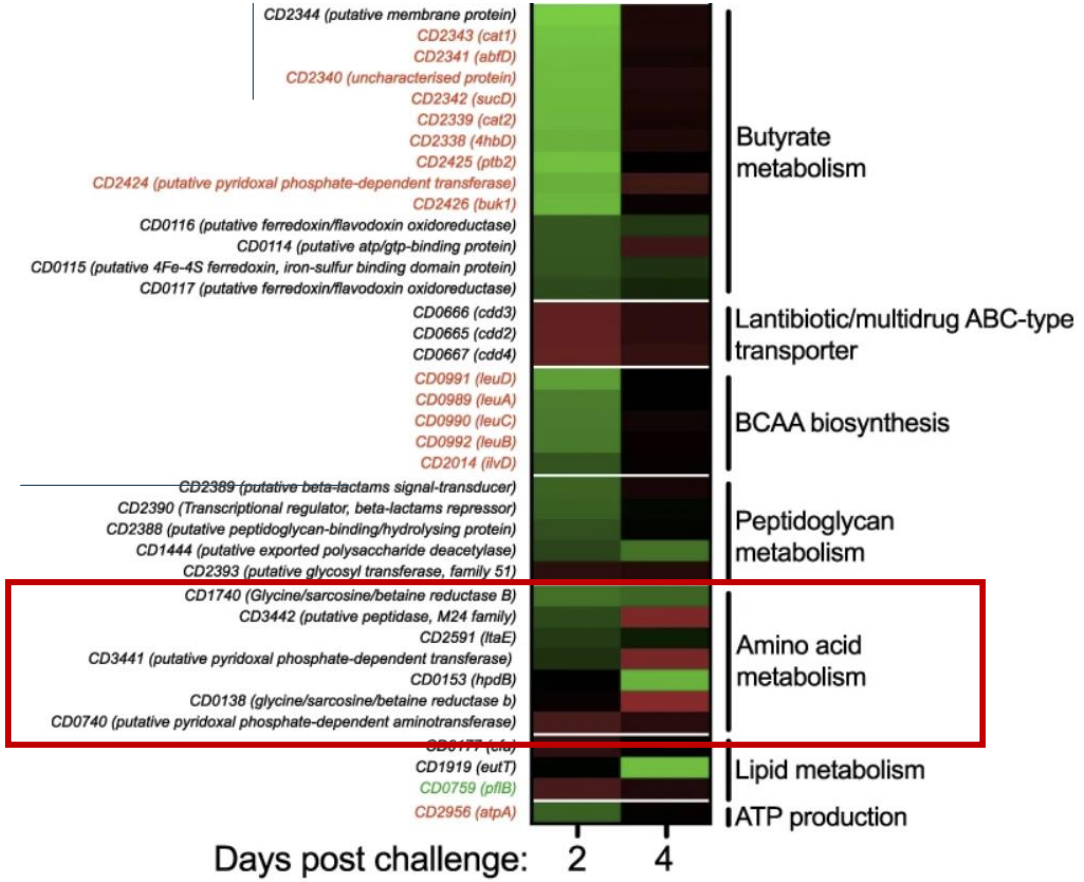
Hypothesis
Toxins induces inflammation, which activates host response and alter pathogen metabolism.

Create tcdR mutant *C. diff*
Fails to produce detectable toxin activity
Doesn’ t elicit significant inflammatory damage to host gut tissue

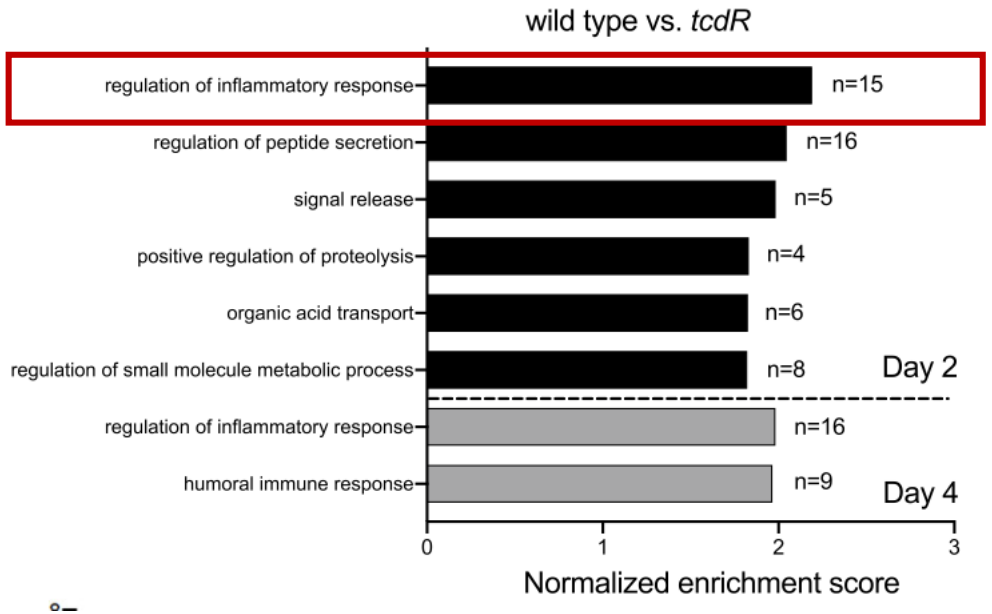


Carbohydrate and amino acid uptake and utilization pathways up-regulated





C. difficile toxin activity induces a highly inflammatory gut environment



Multiple MMPs including Mmp3, Mmp10, Mmp12, and Mmp13 are upregulated

Shen, E., et al. *Commun Biol.* 2020

Summary I

- Reverse genetics: change genotype -> phenotype
- Host side
  - Multiple MMPs are degraded by toxins
- Bacterial side
  - *C. diff* responds by turning on expression of genes that can use these amino acids for growth.

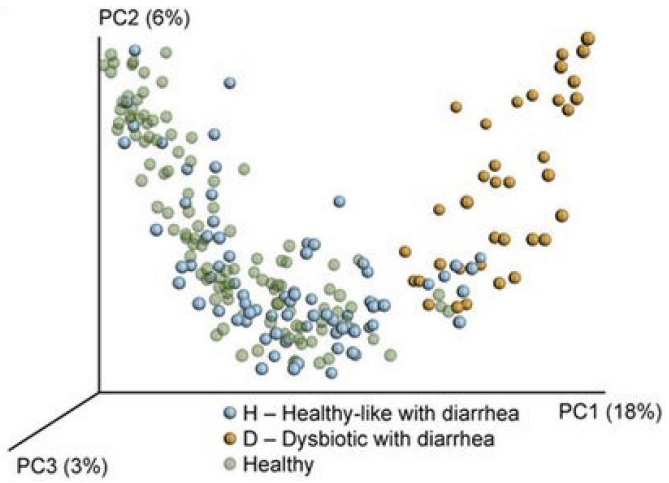
Motivation of study II

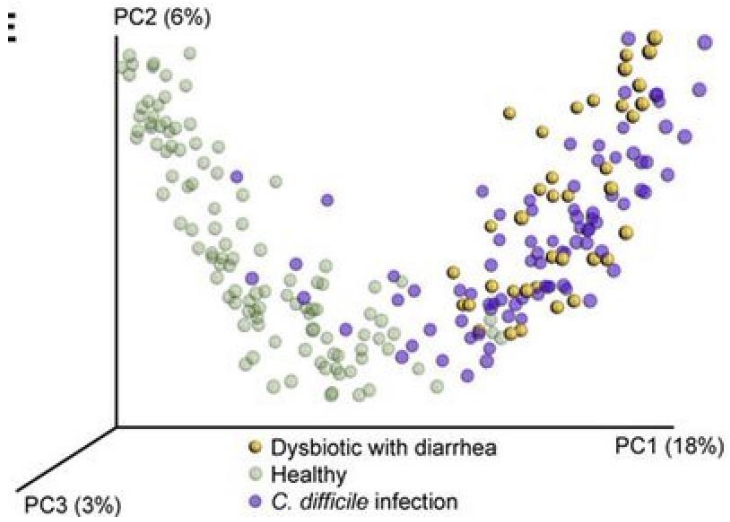
115 patients with diarrhea

Negative for *C. difficile*

~~*C. Difficile* infection → microbial community alteration~~

(Antibiotics →) Preformed microbial community → Increased susceptibility to CDI





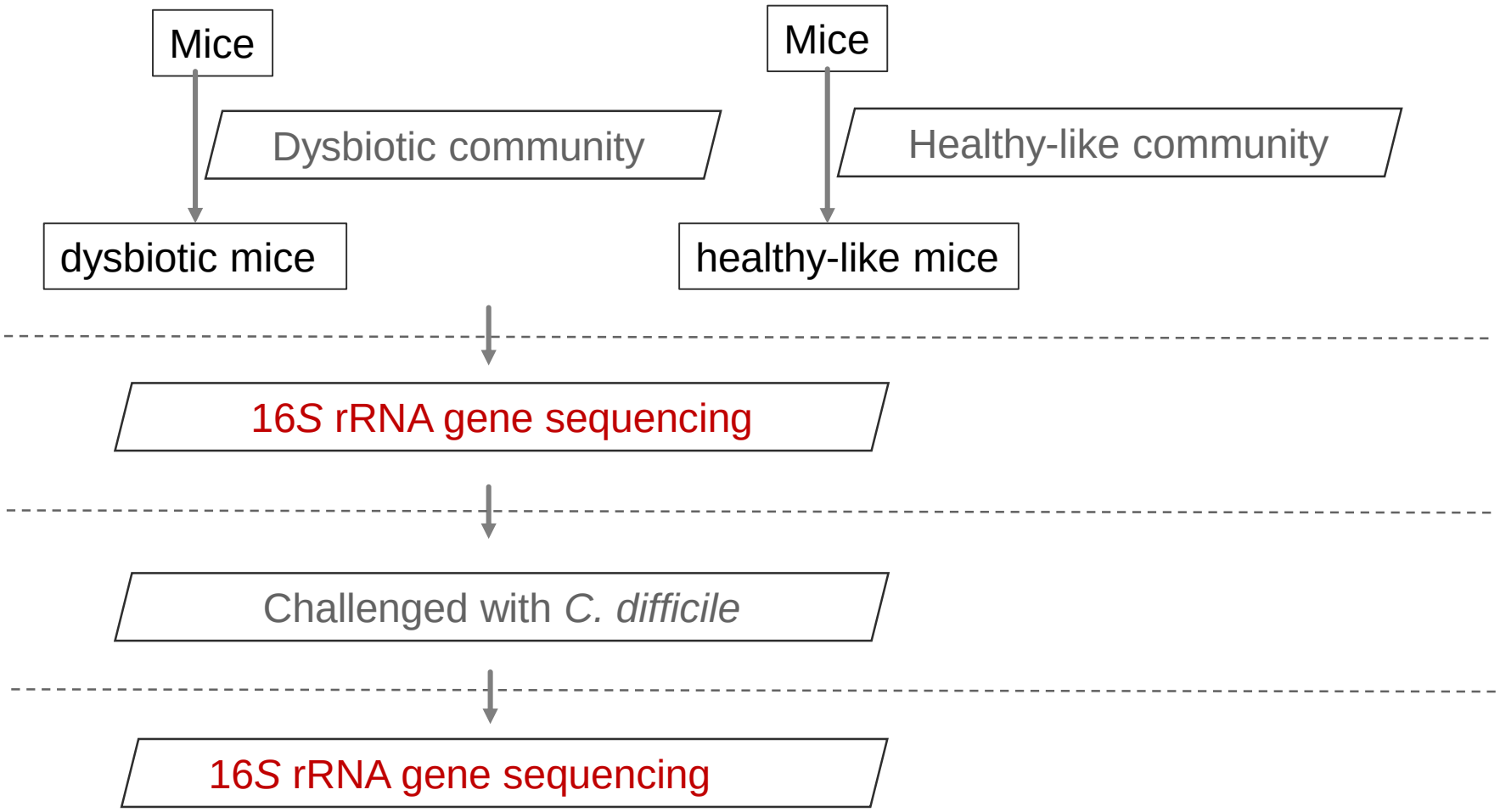
healthy control individuals (*n* = 118)

H: healthy-like diarrhea (*n* = 78)

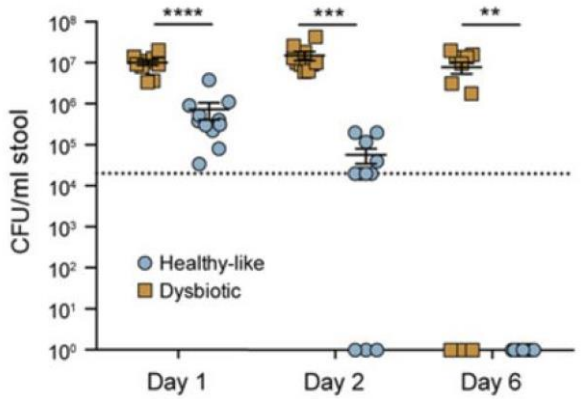
D: dysbiotic with diarrhea (*n* = 37)

C. difficile infection (*n* = 95)

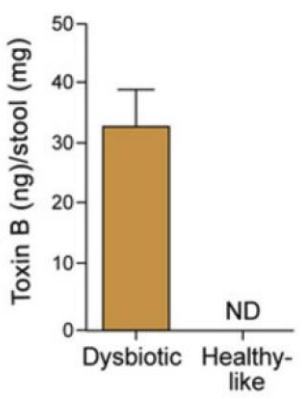
To evaluate community-specific effects on susceptibility to CDI



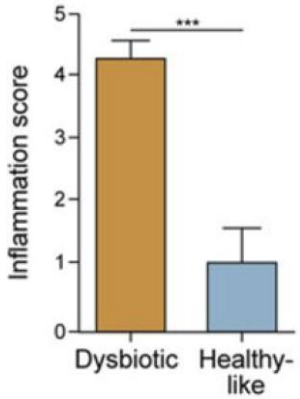
Dysbiotic microbial community → Increased susceptibility to CDI



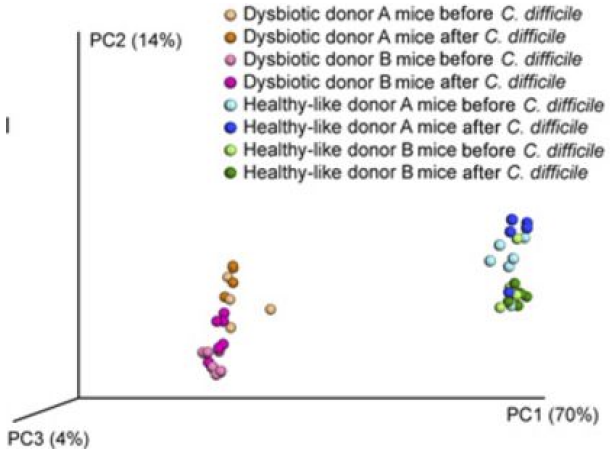
high *C. difficile* loads at dysbiotic mice



increased TcdB toxin level

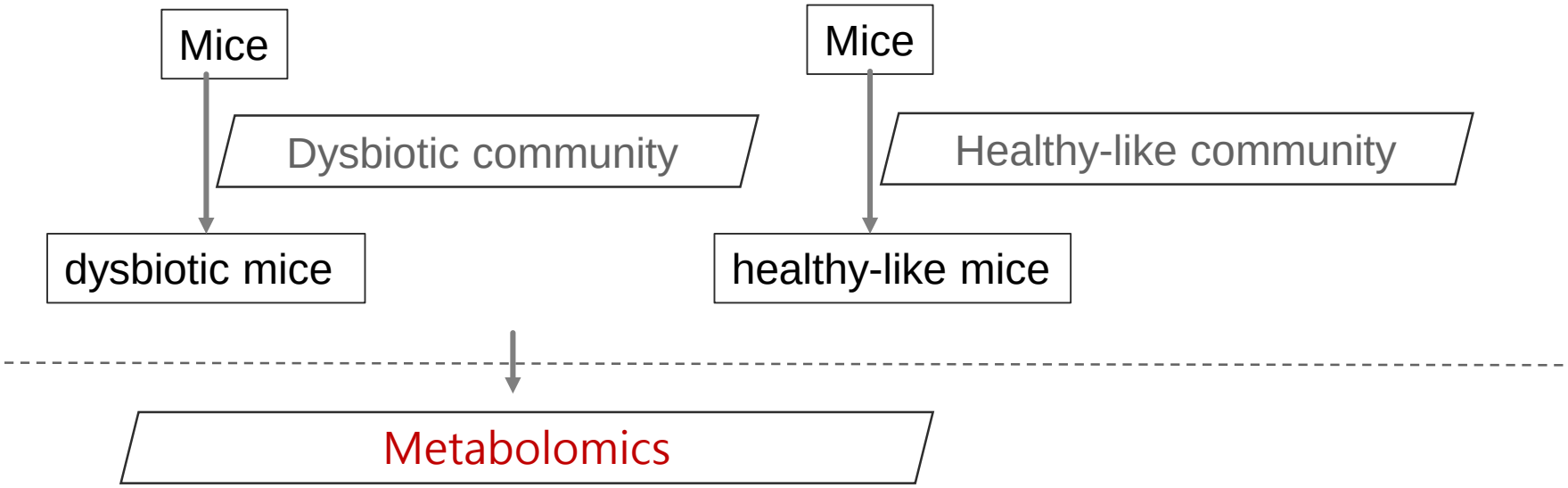


increased inflammation

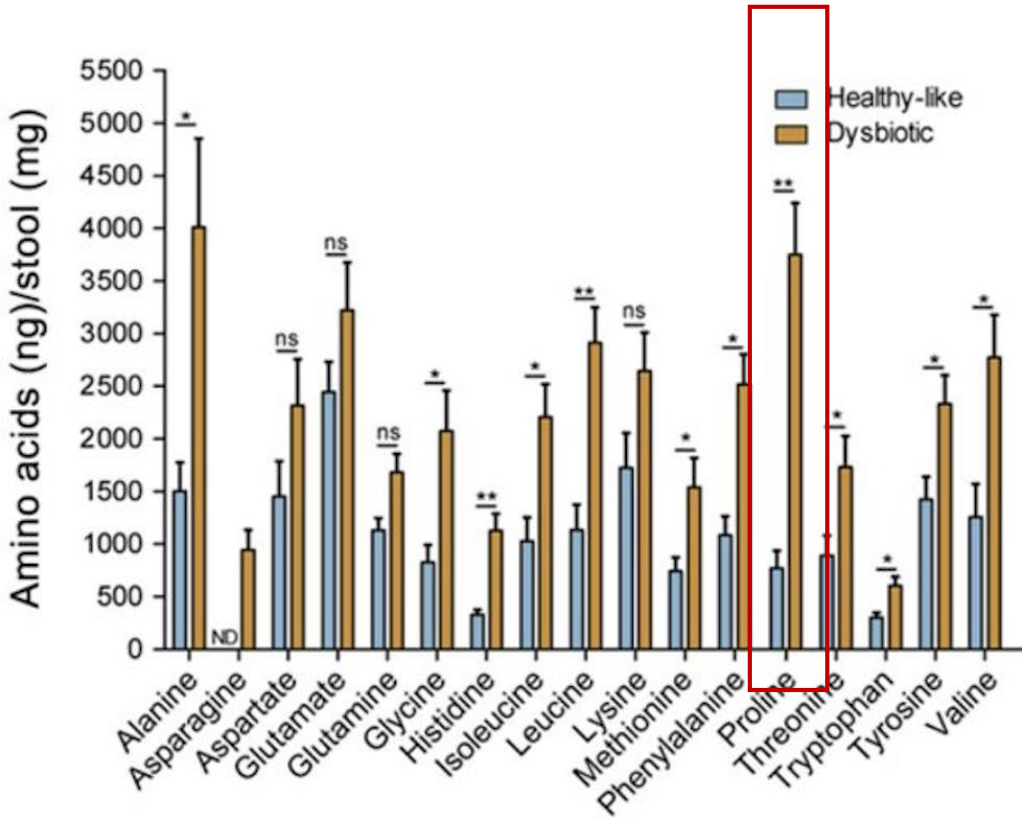


Microbial communities after *C. difficile* challenge were not significantly different from the distance between the microbial communities before and after *C. difficile* challenge

To determine the altered **metabolic states**

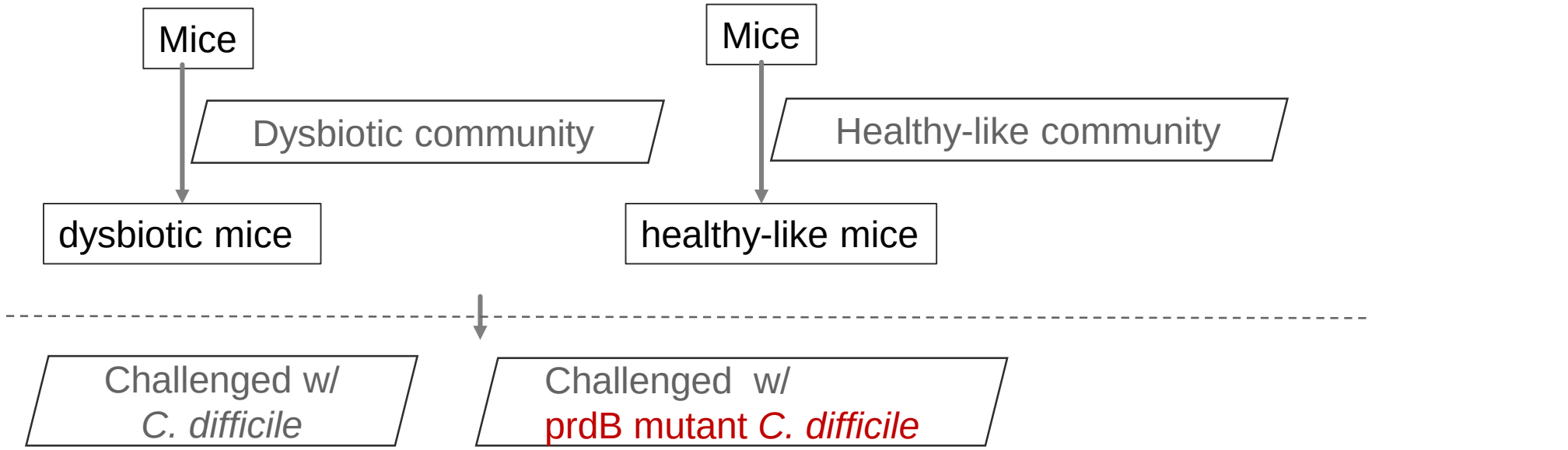


Proline provides a competitive advantage to *C. difficile*



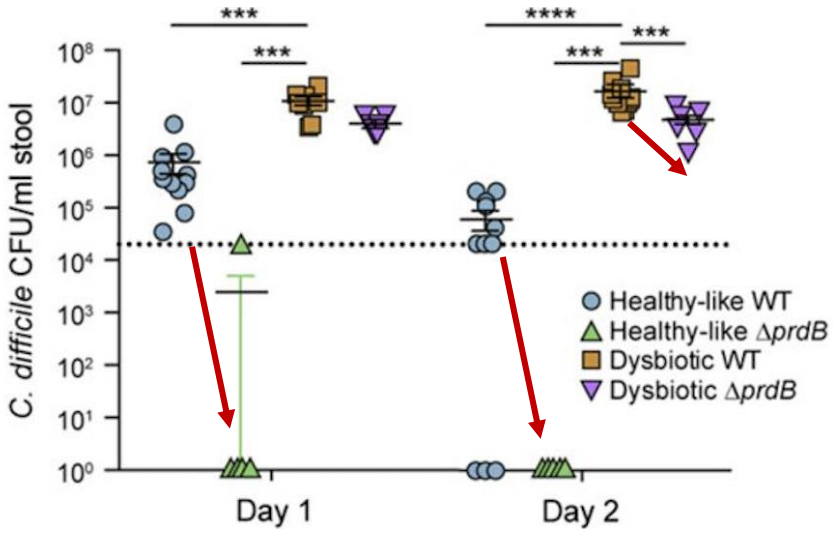
Proline shows the greatest difference between dysbiotic and healthy-like

To determine the relevance of proline for C. difficile colonization

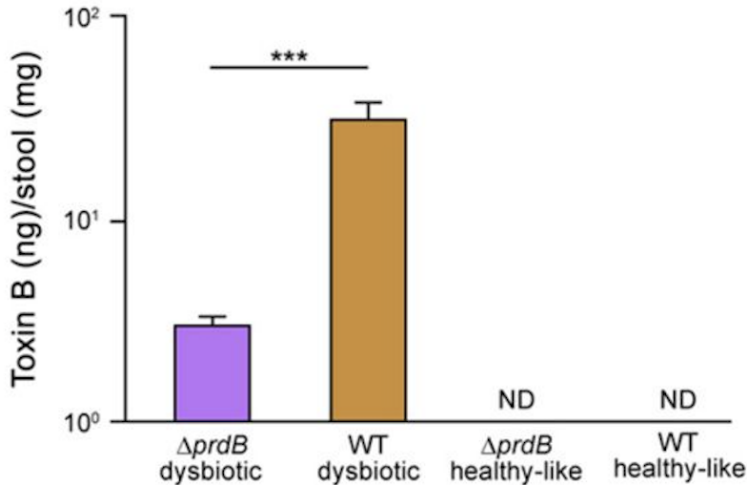


prdB mutant *C. difficile* unable to use proline as an energy source

Proline availability was an important factor governing colonization of C. difficile in dysbiotic mice



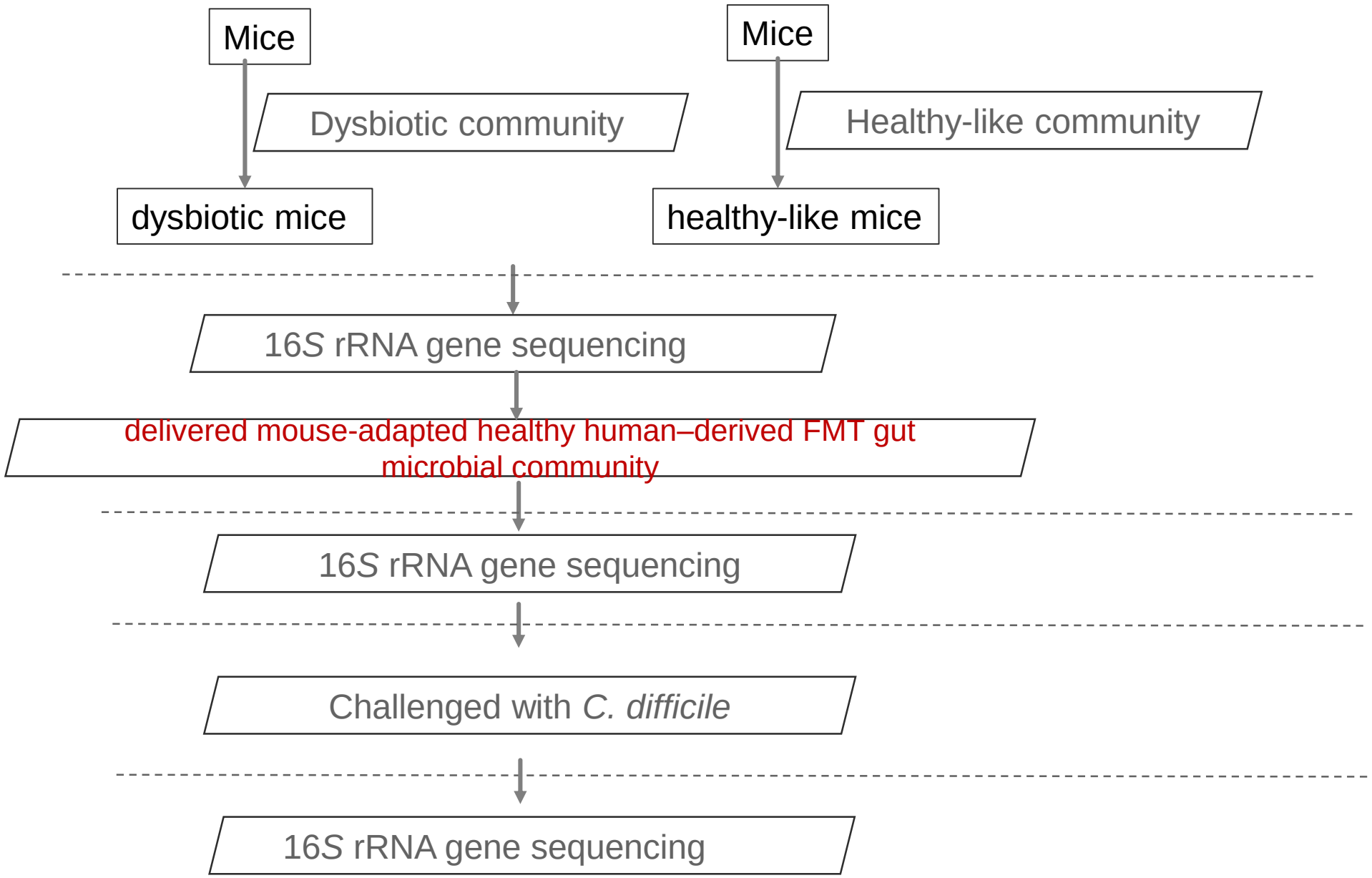
Reduced C. difficile concentration



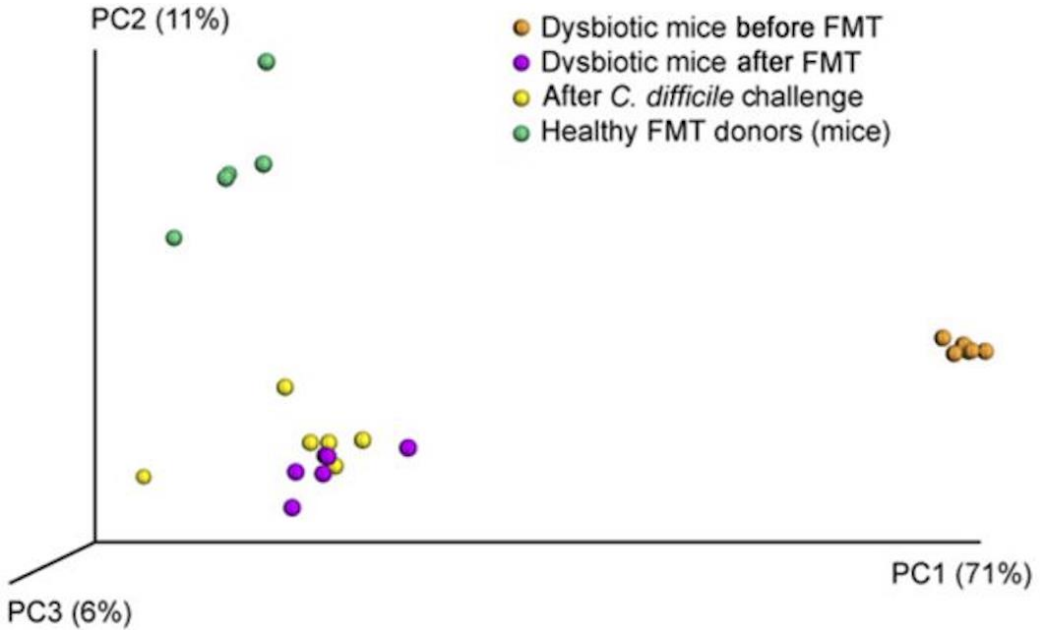
Reduced toxin level

- prdB mutant was undetectable in healthy-like mice at day 1 after challenge
- Significant reduction in dysbiotic mice at day 2

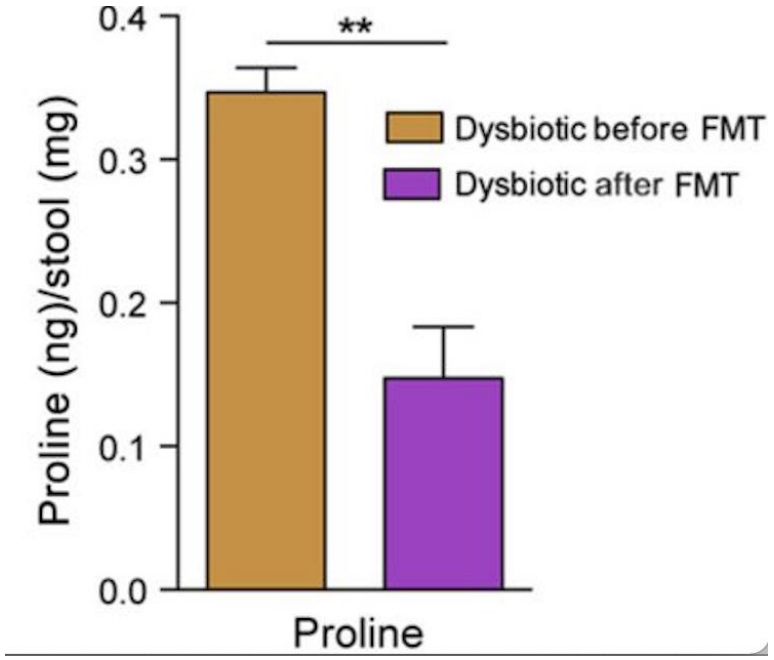
To determine the altered **metabolic states**



FMT reduces free proline and susceptibility to CDI



significant shift in the gut microbial communities of dysbiotic mice to resemble the human fecal donor community after FMT



significant decrease in free proline after FMT

Summary II

- Altered microbe mixes lead to an increase in certain amino acids in the gut, particularly proline
- *C. difficile* can use proline as its main food source, giving it a competitive advantage over microbes that don’t consume the amino acid as readily
- Can explain the following situations:
  - some people are more susceptible to deadly *C. difficile* infections because antibiotic usage disrupt their gut microbial community
  - person might harbor the *C. diff* bacteria in their gut but do not become sick because the beneficial bacteria in their intestine keep it check

Take home message

- *C. difficile* genomes and its toxins are diverse
- *C. difficile* infection includes interactions between host-bacteria and microbe-microbe
- Toxins induce inflammation and host responses. *C. difficile* will also take advantage of the inflammation and altered microbial community
- Next generation sequencing technologies are powerful tools to characterize and analyze CDI related alteration systematically
- Proper experimental design is necessary in order to incorporate sequencing data into the study

References

Avican, K., Aldahdooh, J., Togninalli, M., Mahmud, A.K.M.F., Tang, J., Borgwardt, K.M., Rhen, M., and Fällman, M. (2021). RNA atlas of human bacterial pathogens uncovers stress dynamics linked to infection. Nat Commun *12*, 3282.

Byrd, A.L., and Segre, J.A. (2016). Adapting Koch's postulates. Science *351*, 224–226.

Chaitankar, V., Karakülah, G., Ratnapriya, R., Giuste, F.O., Brooks, M.J., and Swaroop, A. (2016). Next generation sequencing technology and genomewide data analysis: Perspectives for retinal research. Progress in Retinal and Eye Research *55*, 1–31.

Cornforth, D.M., Dees, J.L., Ibberson, C.B., Huse, H.K., Mathiesen, I.H., Kirketerp-Møller, K., Wolcott, R.D., Rumbaugh, K.P., Bjarnsholt, T., and Whiteley, M. (2018). Pseudomonas aeruginosa transcriptome during human infection. PNAS *115*, E5125–E5134.

Costa, T.R.D., Felisberto-Rodrigues, C., Meir, A., Prevost, M.S., Redzej, A., Trokter, M., and Waksman, G. (2015). Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat Rev Microbiol *13*, 343–359.

Di Lonardo, A., Nasi, S., and Pulciani, S. (2015). Cancer: We Should Not Forget The Past. J. Cancer *6*, 29–39.

Falkow, S. (1988). Molecular Koch's postulates applied to microbial pathogenicity. Rev Infect Dis *10 Suppl 2*, S274-276.

Godaly, G., and Svanborg, C. (2007). Urinary tract infections revisited. Kidney International *71*, 721–723.

Leyton, D.L., Rossiter, A.E., and Henderson, I.R. (2012). From self sufficiency to dependence: mechanisms and factors important for autotransporter biogenesis. Nat Rev Microbiol *10*, 213–225.

Lloyd-Price, J., Arze, C., Ananthakrishnan, A.N., Schirmer, M., Avila-Pacheco, J., Poon, T.W., Andrews, E., Ajami, N.J., Bonham, K.S., Brislawn, C.J., et al. (2019). Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature *569*, 655–662.

Wong, S.H., and Yu, J. (2019). Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nat Rev Gastroenterol Hepatol *16*, 690–704.

THANK YOU