How Does Fecal Microbiota Transplantation Treat *Clostridium difficile* Infection?

Alexander Khoruts, MD

University of Minnesota

Human Microbiome Science 2013

Disclosures

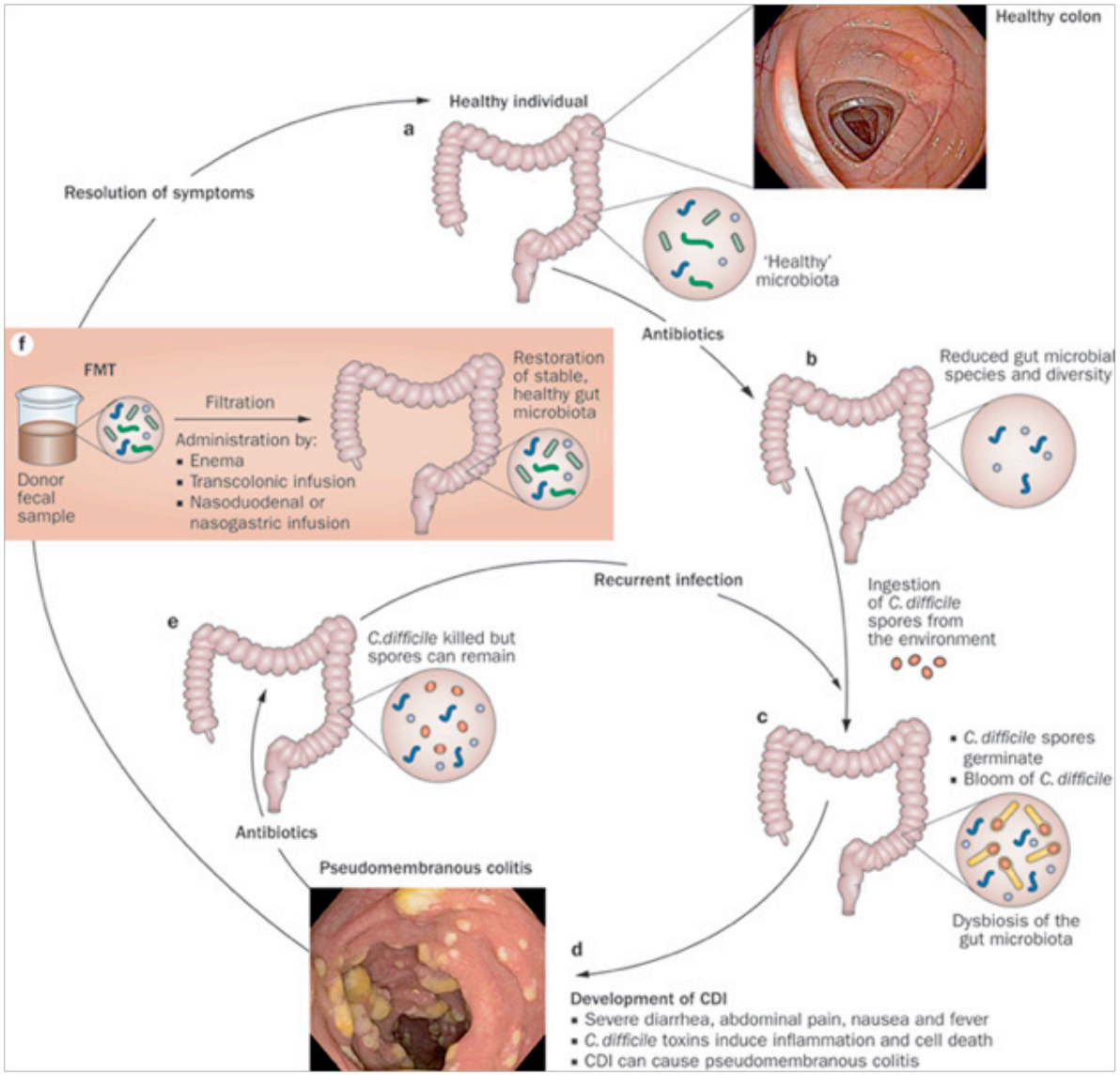
- Research funding from CIPAC Limited, a start-up company commercializing Full-spectrum Microbiota for FMT

- NIH and UMN funding for studies of gut microbiota following FMT

Clinical Case

61 year old woman referred for evaluation of chronic diarrhea for 8 months. Symptoms originally started following treatment with cephalosporin and quinolone antibiotics for back surgery and pulmonary infection. During these 8 months she had several hospitalizations for intravenous hydration. A colonoscopy showed "ischemic colitis" on biopsies. Intermittently she was treated with variable success with Metronidazole and Vancomycin. She had bowel movements every 15 minutes with urgency and tenesmus. She lost 27 kg of weight and was confined to a wheelchair.

Recurrent *C. difficile* Infection Syndrome



Borody and Khoruts, 2011

Fecal Microbiota Transplantation: Mechanisms

Ryan Pequin, Three Word Phrase

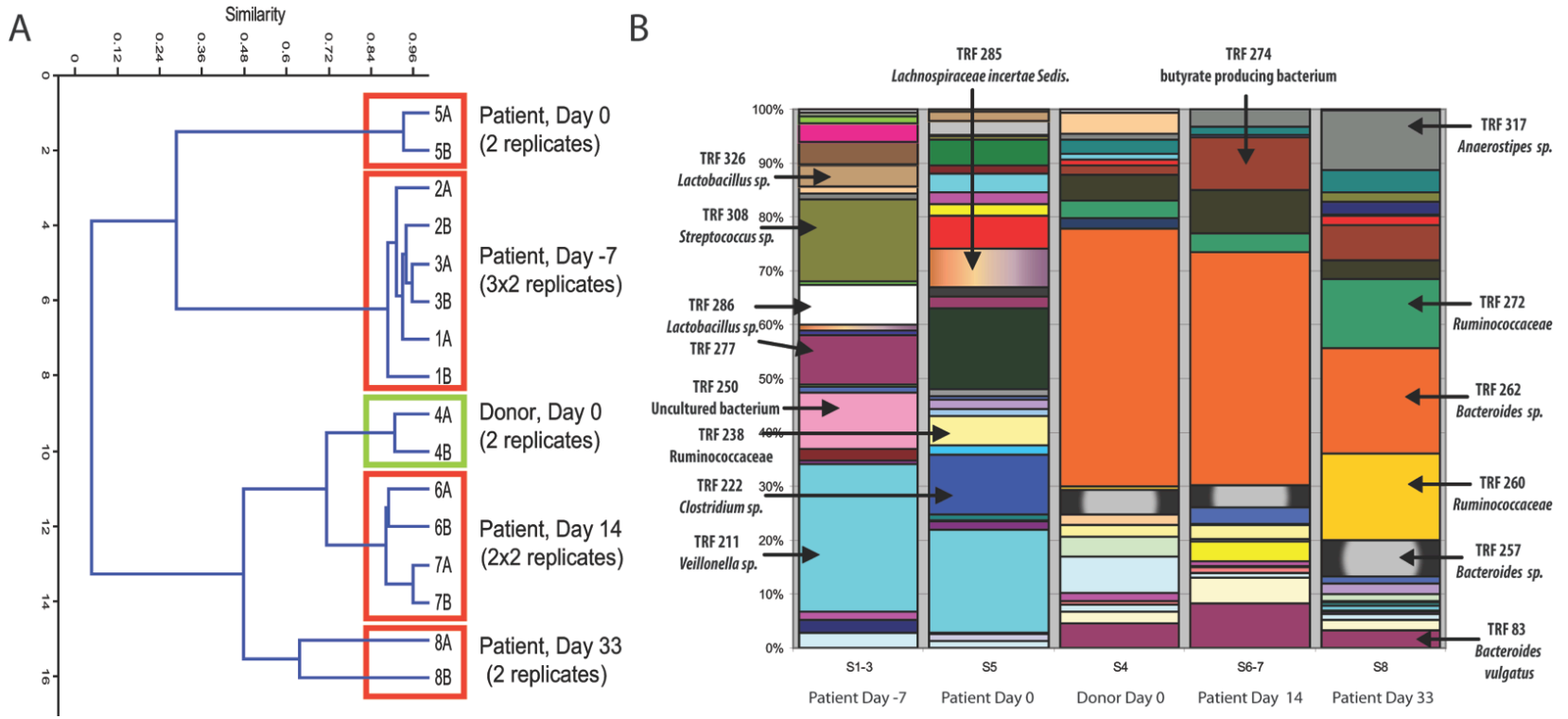
Dr. Ben Eiseman (1917-2012)

- Chief of Surgery, Denver VA Hospital, 1953-61
- Founding Chairman of Surgery, University of Kentucky, 1961-67
- Founding Chairman of Surgery, Denver General Hospital, 1967-77
- > 450 scientific articles
- 7 books
- Active Military Duty in 4 wars
- Rear Admiral (MC) USNR – retired 1974
- Active academic through 2012

Dr. Ben Eiseman (1917-2012)

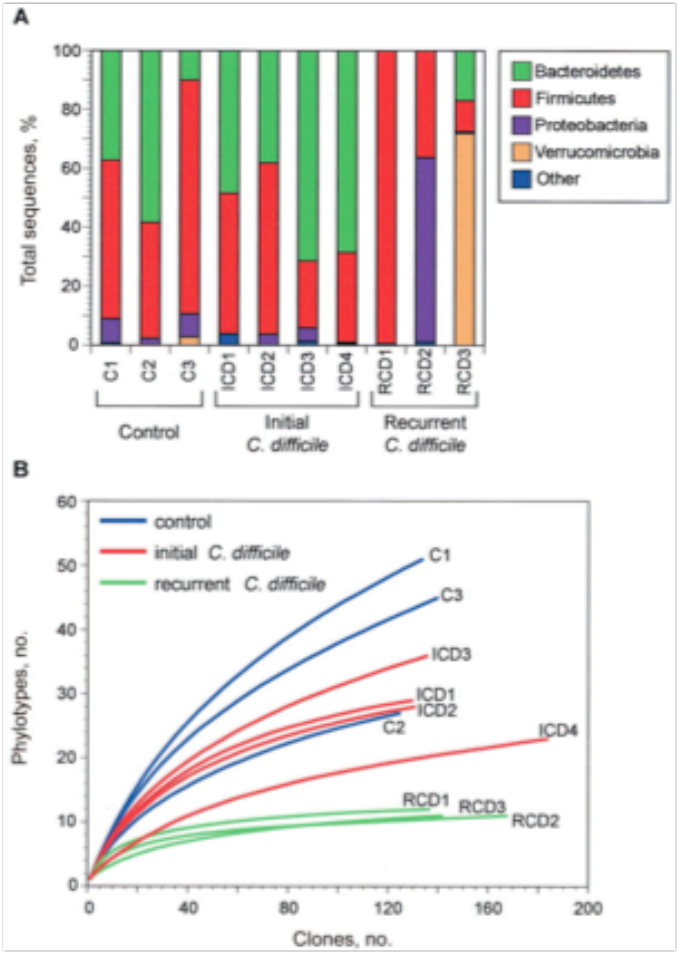
"In the early days of oral antibiotics we were plagued by frequent diarrhea in our patients due presumably to killing off intestinal bacteria. I was Chief of Surgery at the VA and simplistically considered merely reintroducing normal organisms to counter such absence. Those were days when if one had an idea, we simply tried it. It seemed to work and I wrote it up. It made a small splash…Best wishes. Ben Eiseman Emeritus Professor of Surgery – Now age 93" (2012).

Clinical Case



Khoruts, et al., 2010

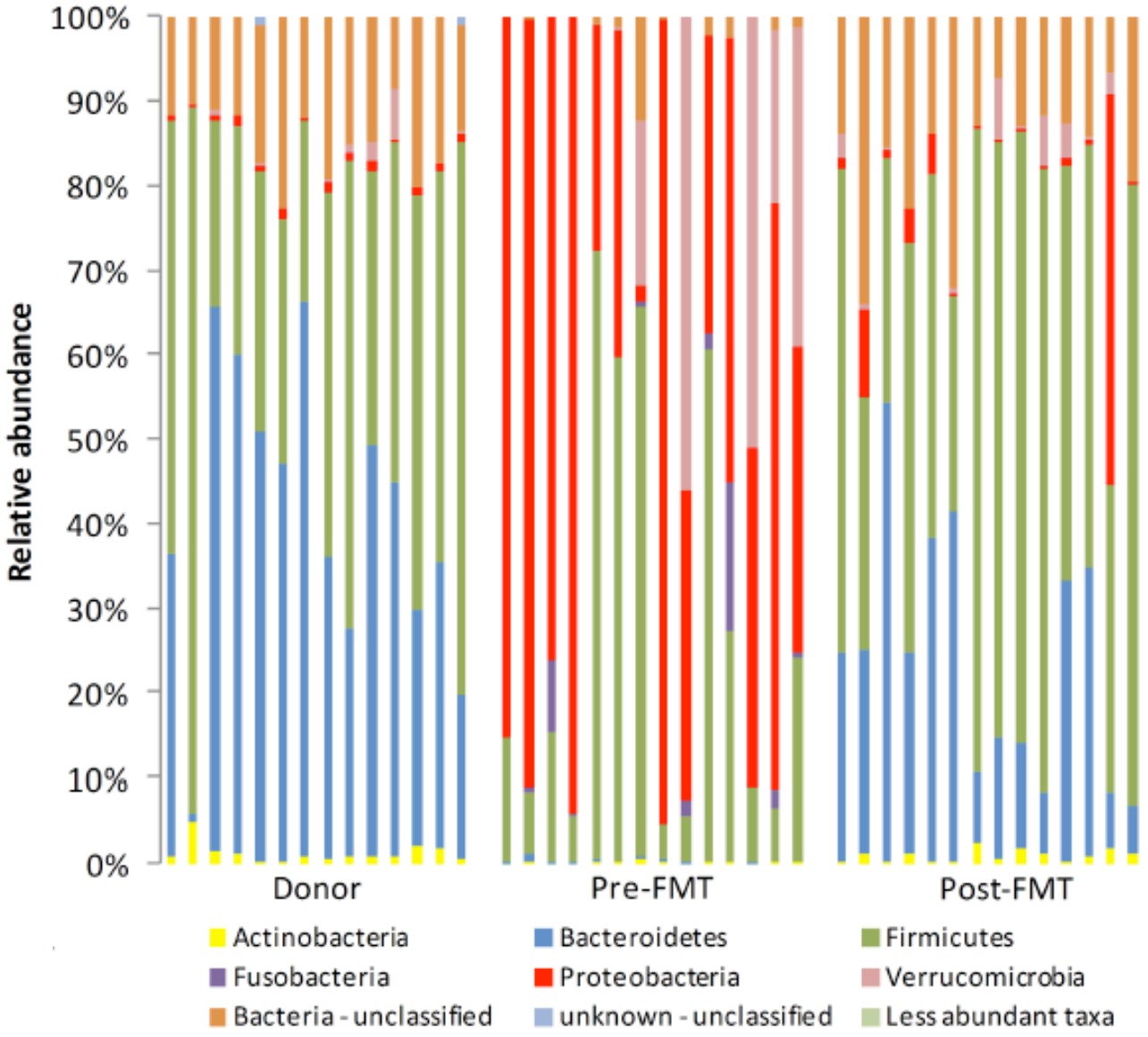
Analysis of 16S clone libraries of the fecal microbiota in patients with antibiotic-associated diarrhea due to Clostridium difficile.



Chang J Y et al. J Infect Dis. 2008;197:435-438

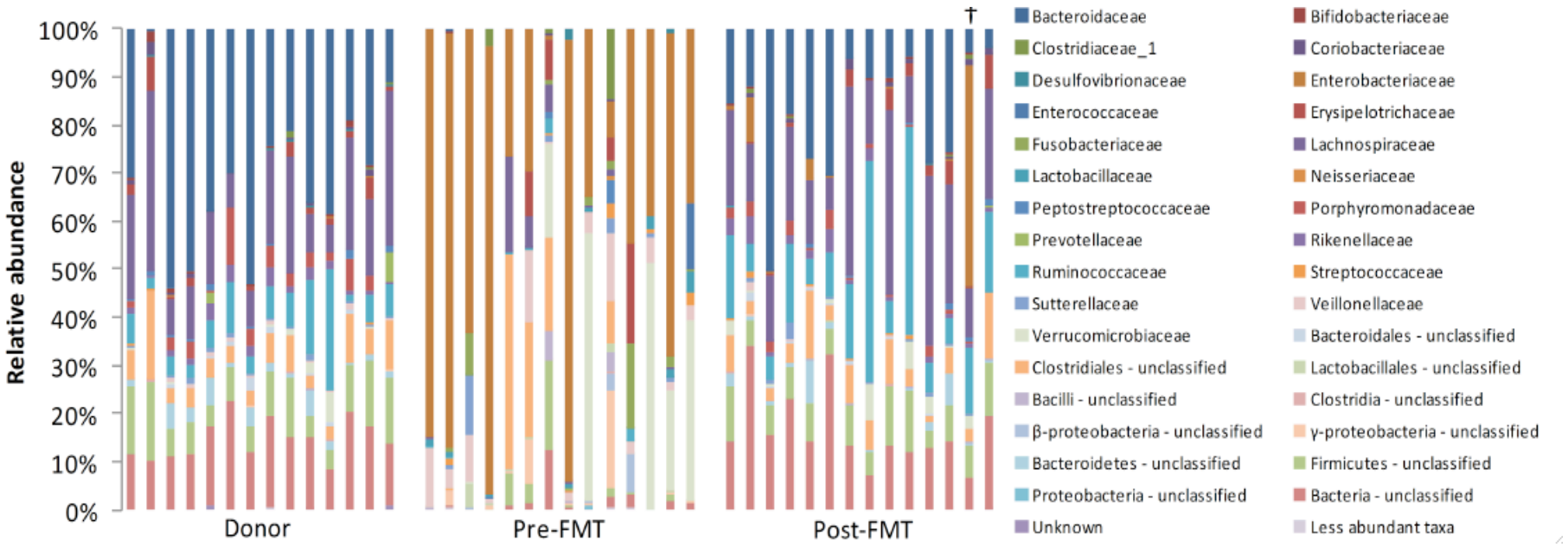
The Journal of Infectious Diseases

FMT Results in Restoration of Gut Microbial Diversity



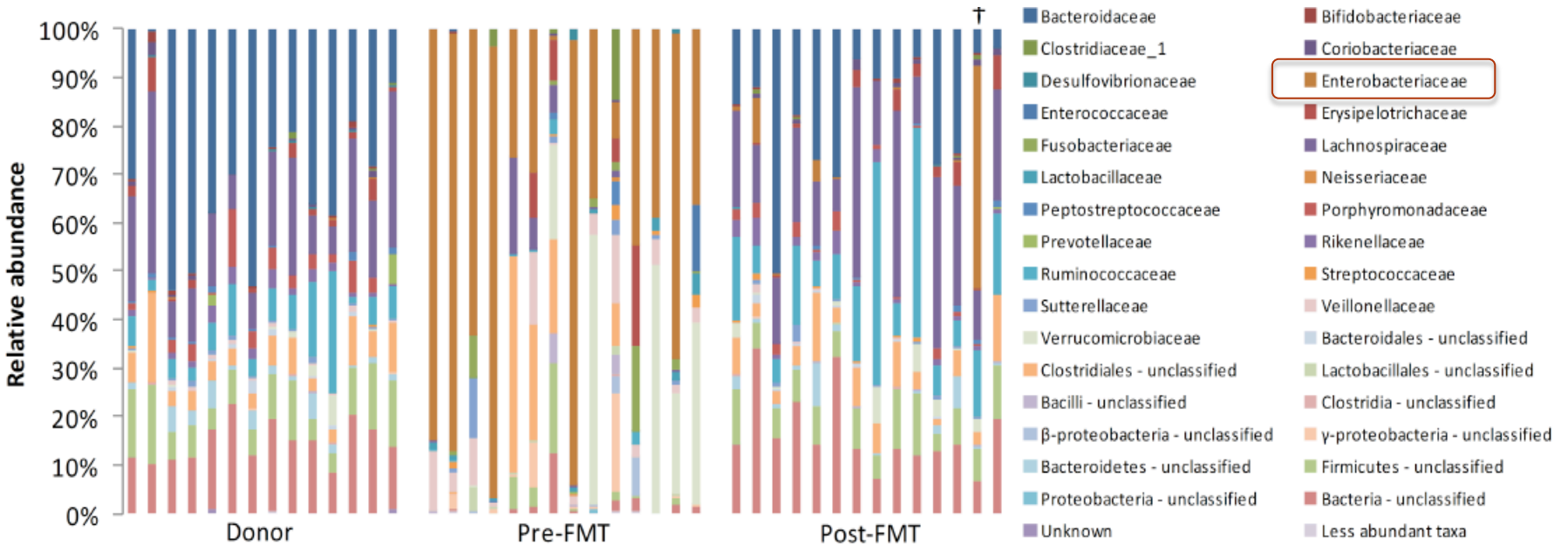
Weingarden et al., Poster #45

FMT Results in Restoration of Gut Microbial Diversity



Weingarden et al., Poster #45

FMT Results in Restoration of Gut Microbial Diversity



Weingarden et al., Poster #45

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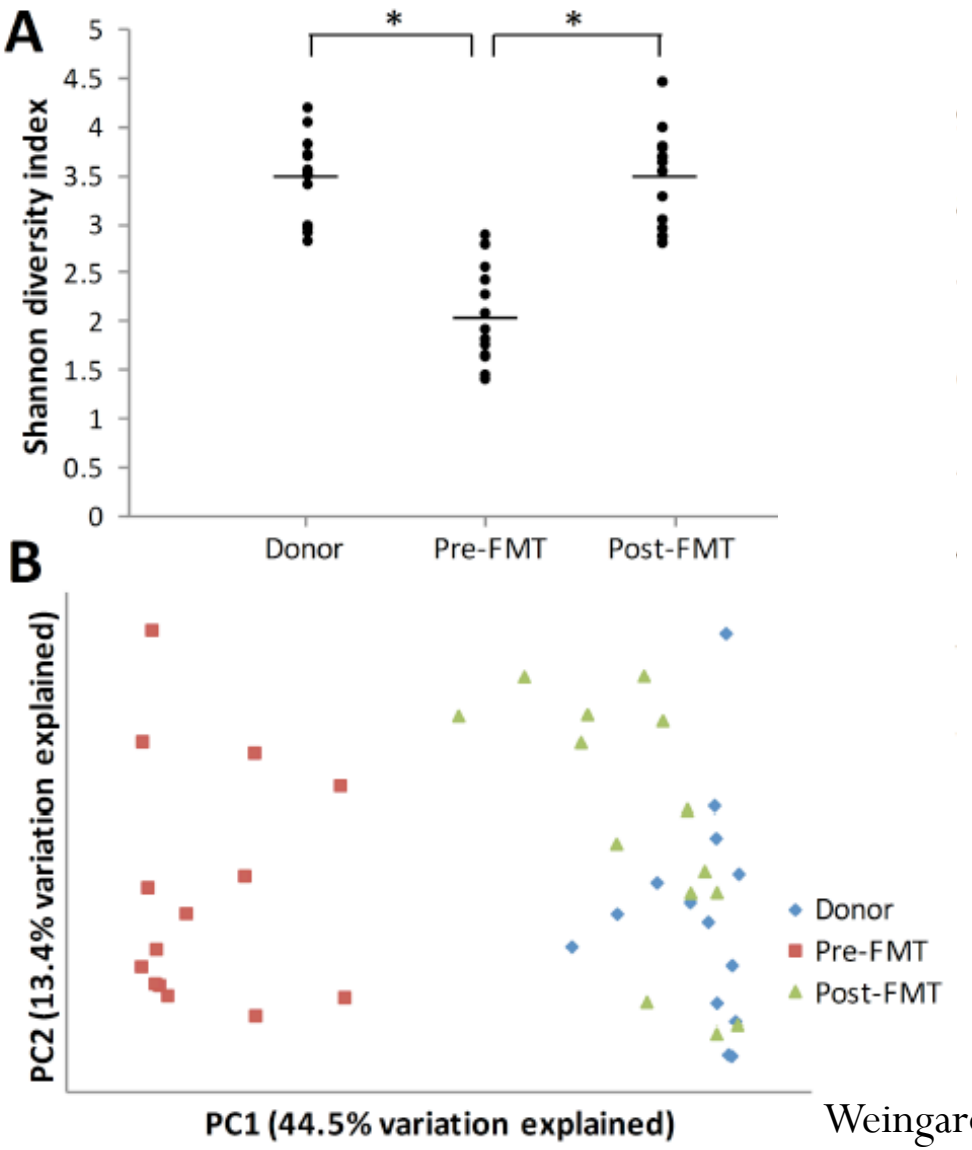

Weingarden et al., Poster #45

What about the “Yuck” factor?

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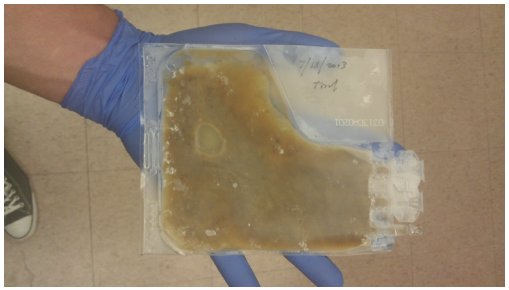

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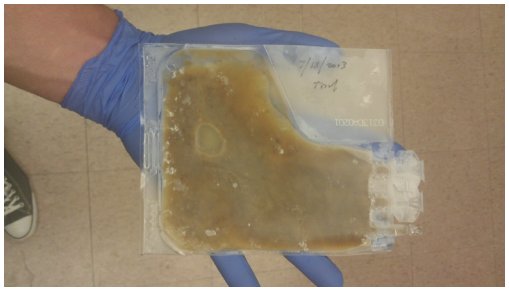
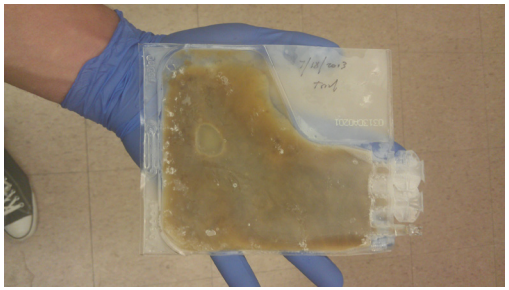


Standardized Full Spectrum Microbiota

- Rigorously tested volunteer donors
- Cryopreserved
- Virtually eliminated odor
- The same number of bacteria per dose
- Manufactured under GMP conditions at an FDA registered facility at the UMN

What about the “Yuck” factor?



Potential Mechanisms of FMT in R-CDI

Microbiota

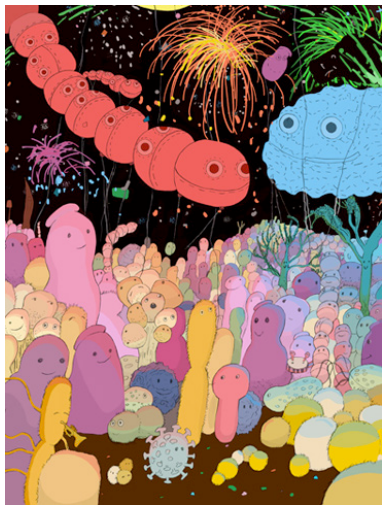
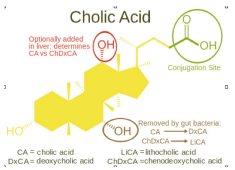


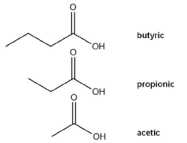
Illustration by Adam Alaniz

Host

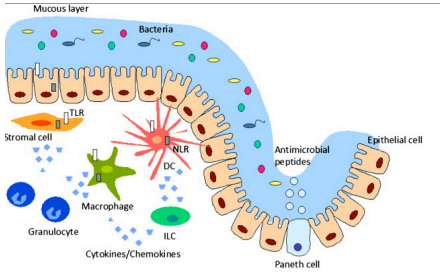
Bile Acids



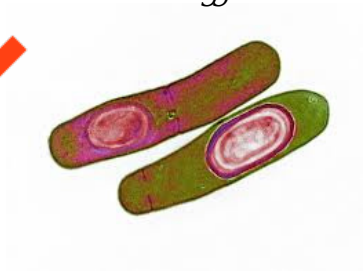
SCFA



Innate and Adaptive Immunity



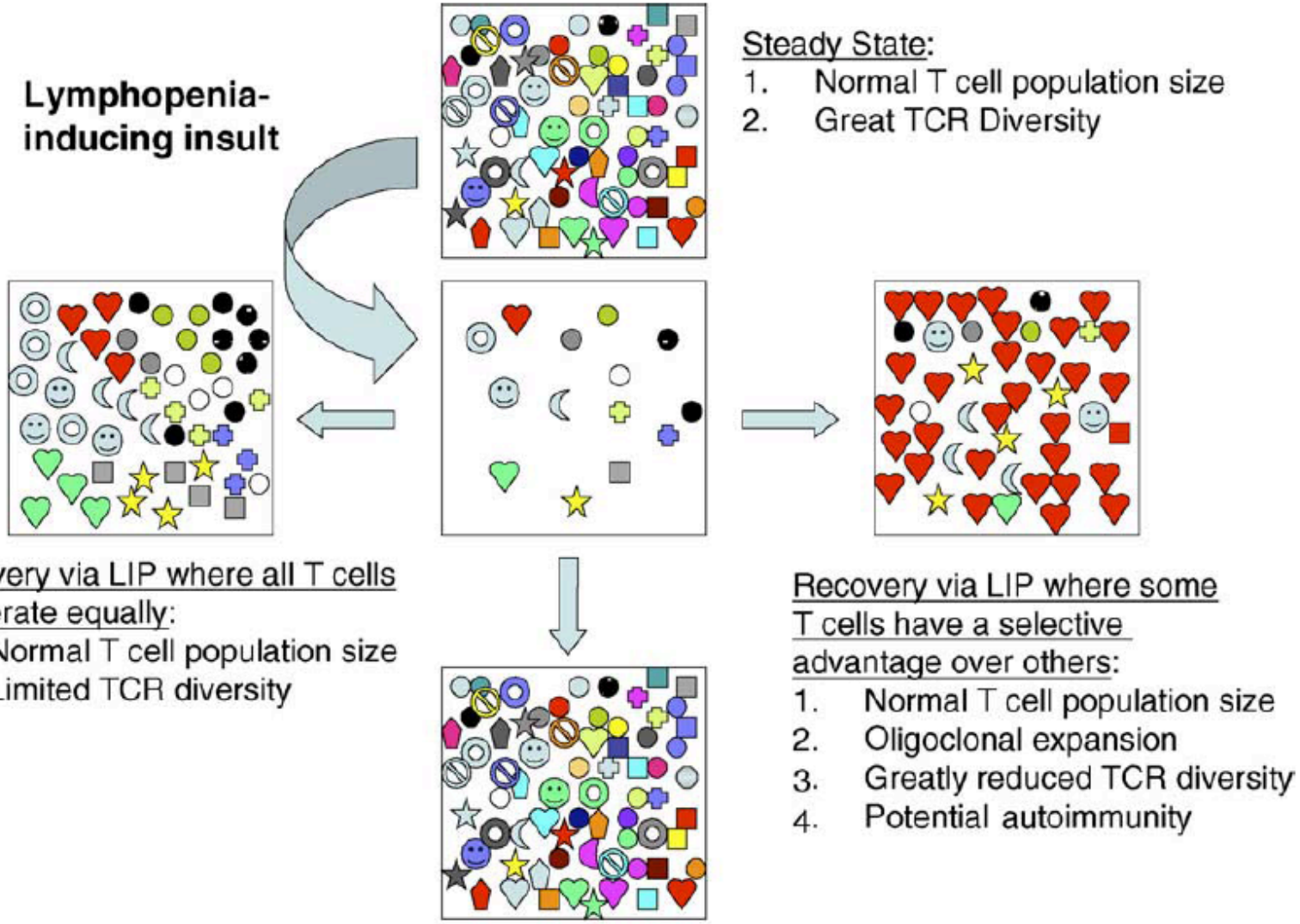
C. difficile



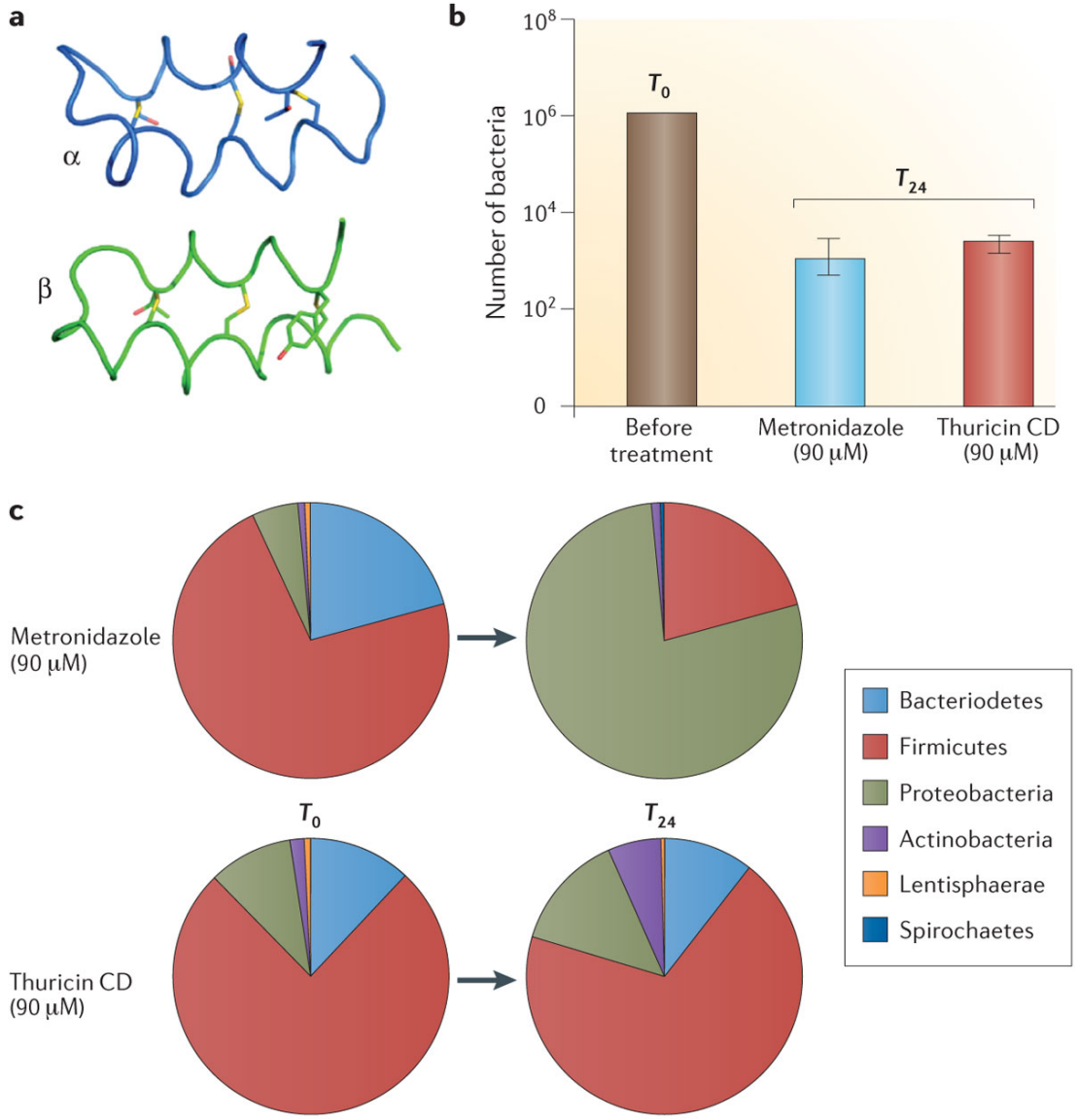
- Sporulation
- Spore germination
- Vegetative growth
- Adhesion to epithelial cells
- Toxin production

Competitive Niche Exclusion

Illustration by Adam Alaniz



Khoruts A and Fraser JM (2005)

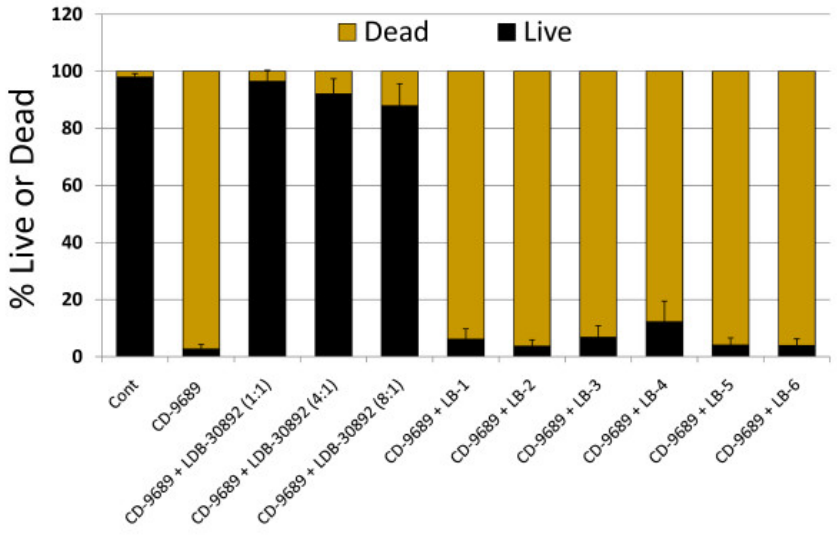


- Thuricin CD inhibits vegetative growth of *C. difficile* in an ex vivo colon infection model
- Produced by *B. thuringiensis*
- Narrow activity spectrum

Rea et al., *PNAS* (2010)

Cotter et al. (2013)

Lactobacillus delbrueckii ssp. Bulgaricus B-30892 inhibits cytotoxicity and adhesion of *C. difficile* to Caco-2 cells



LDB releases bioactive components that inhibit *C. difficile* blocks toxin activity (mechanism?)

Banerjee, P, et al., (2009)

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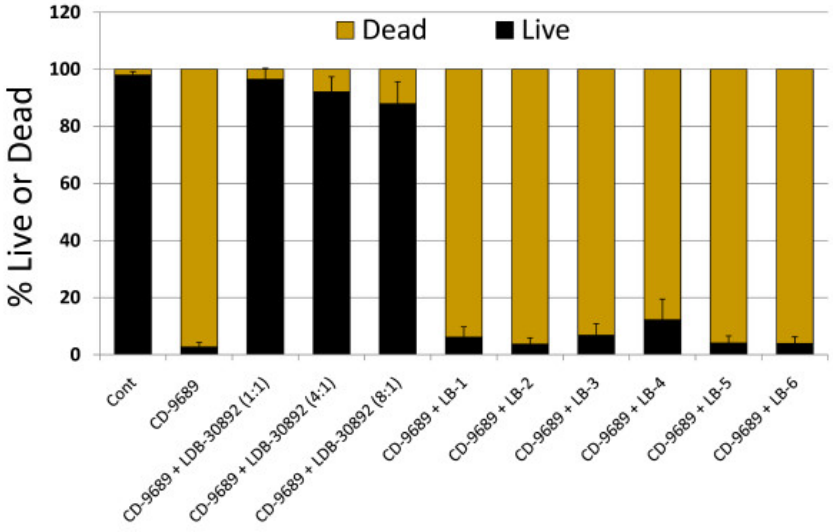

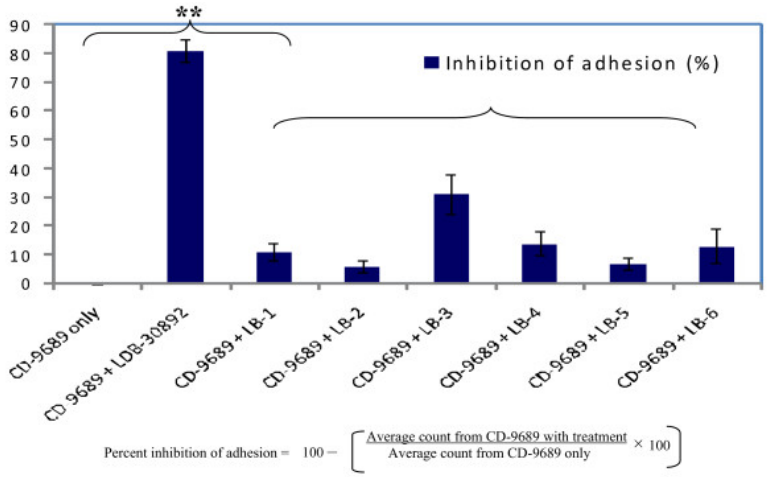

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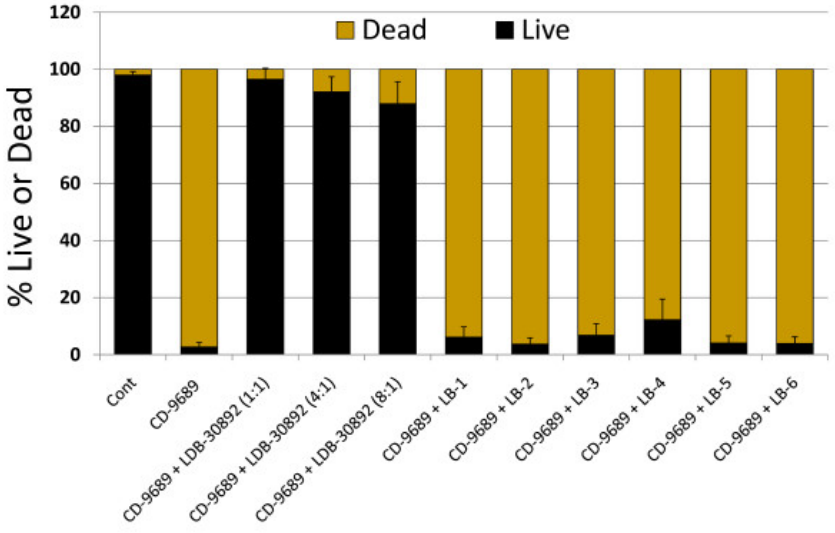
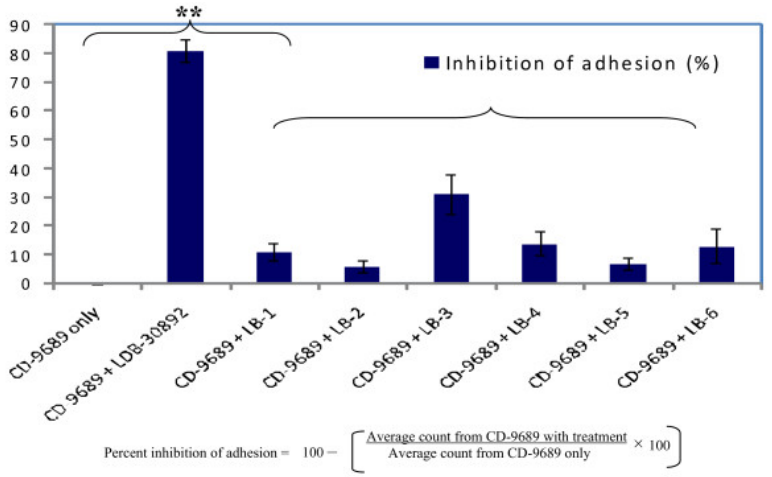
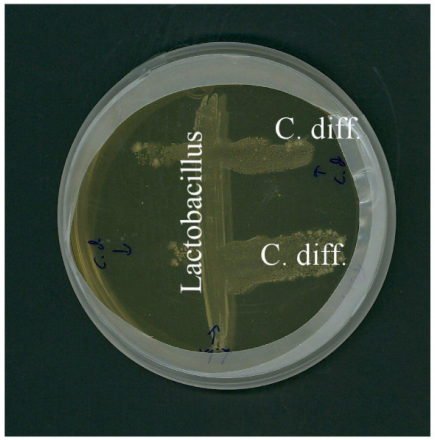




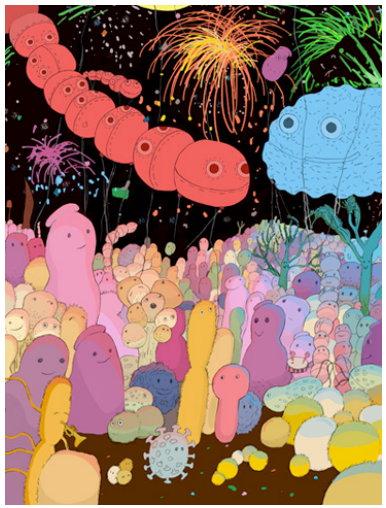


LDB releases bioactive components that inhibit *C. difficile* blocks toxin activity (mechanism?) and adhesion to epithelial cells, but vegetative growth of *C. difficile* is not impaired.

Banerjee P, et al., (2009)

Immune-mediated colonization resistance

Microbiota



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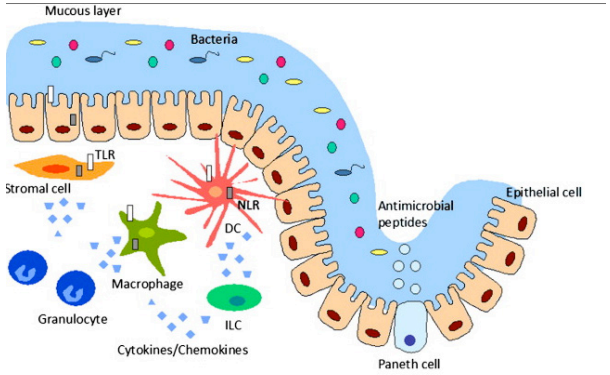
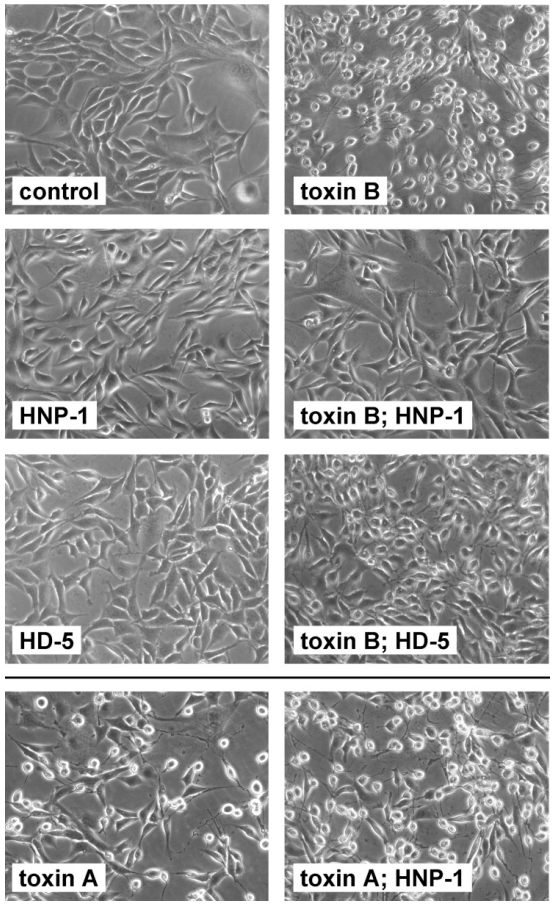


Immunity against *C. difficile*



- Antimicrobial peptides

α-Defensins neutralize *C. difficile* toxin B

Giesemann T, et al. (20008)

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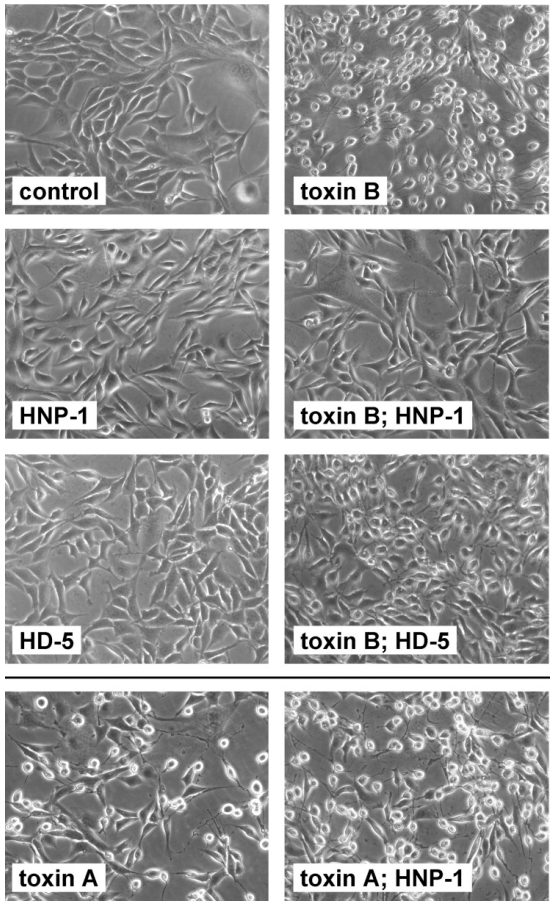

α-Defensins neutralize *C. difficile* toxin B

- Antimicrobial peptides
- NOD1
- MyD88
- IL-1β
- CXCL1
- TLR5
- Anti-*C. difficile* toxin IgG

Giesemann T, et al. (20008)
Hasegawa M, et al. (2011)
Jarchum I, et al. (2012)
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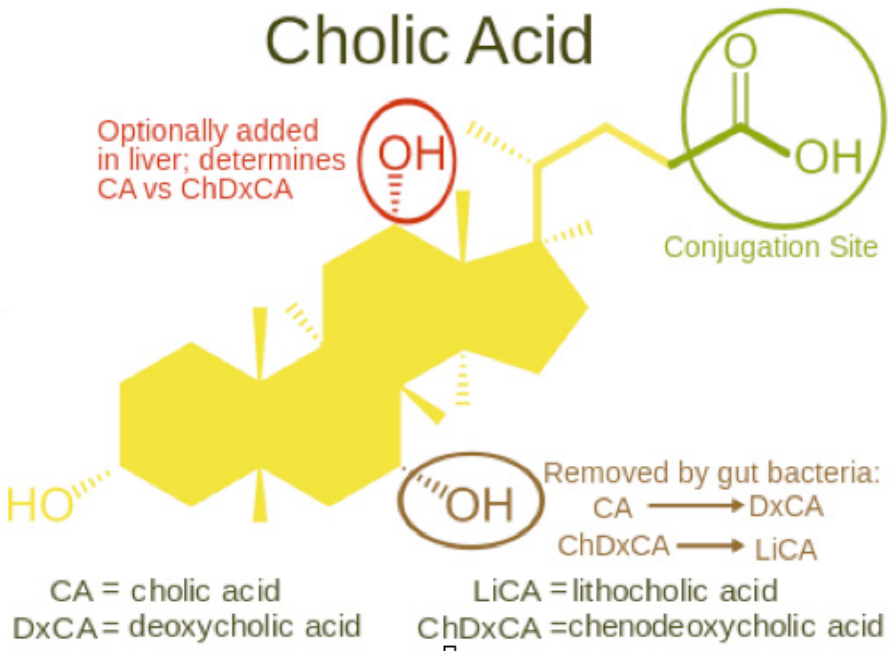
Kyne L, et al. (2000)
Lowy I, et al. (2010)

Bile Acids and Salts



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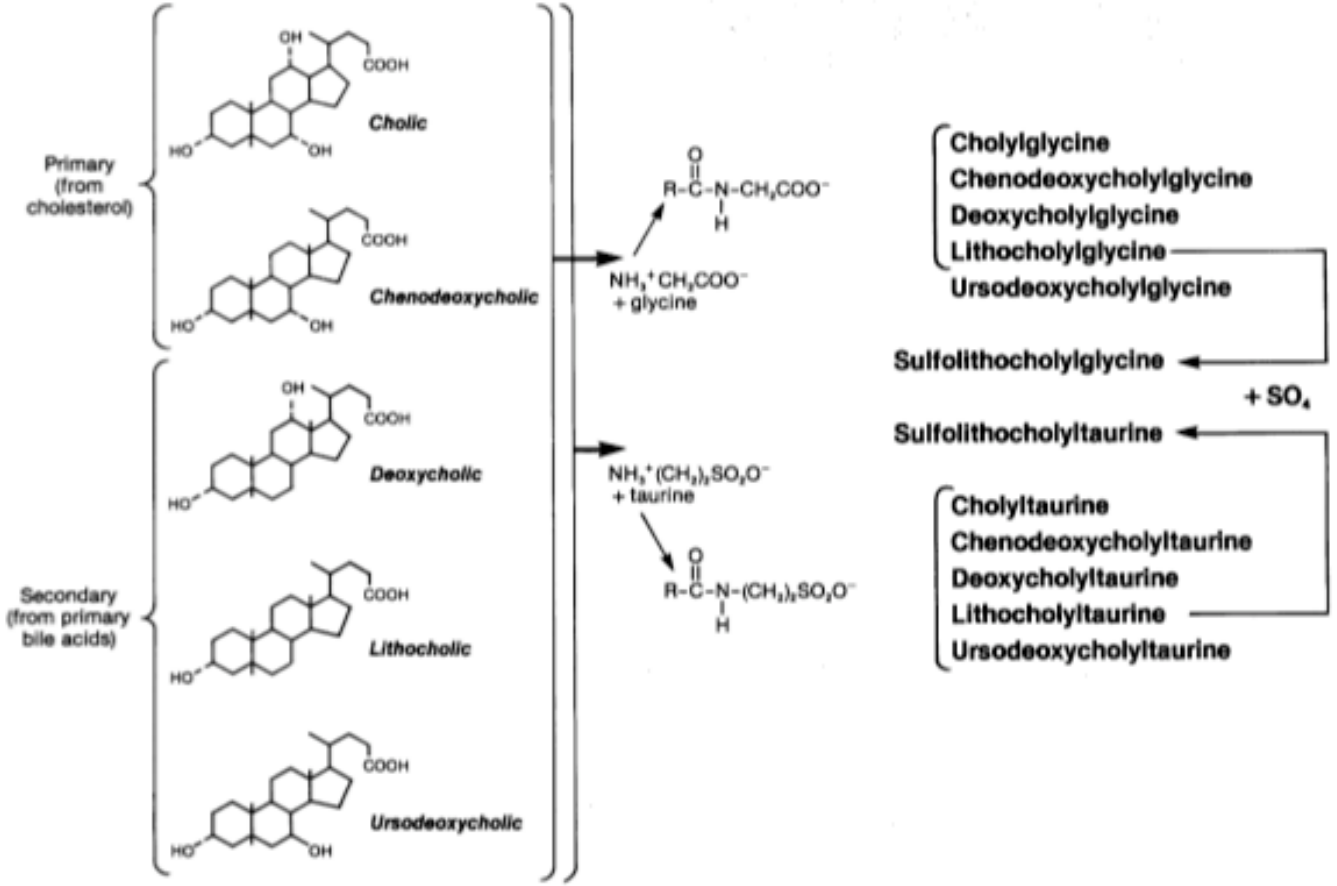
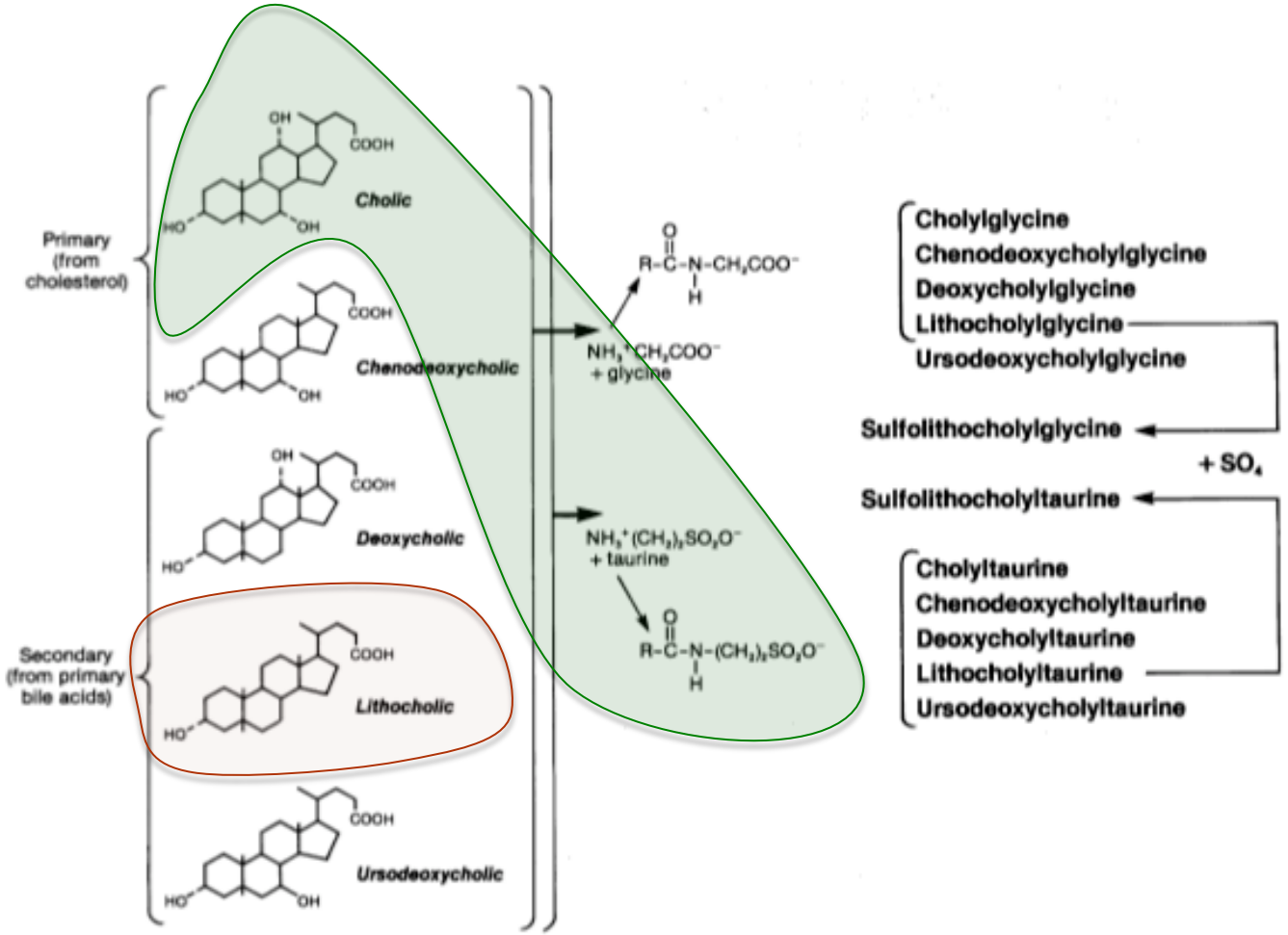


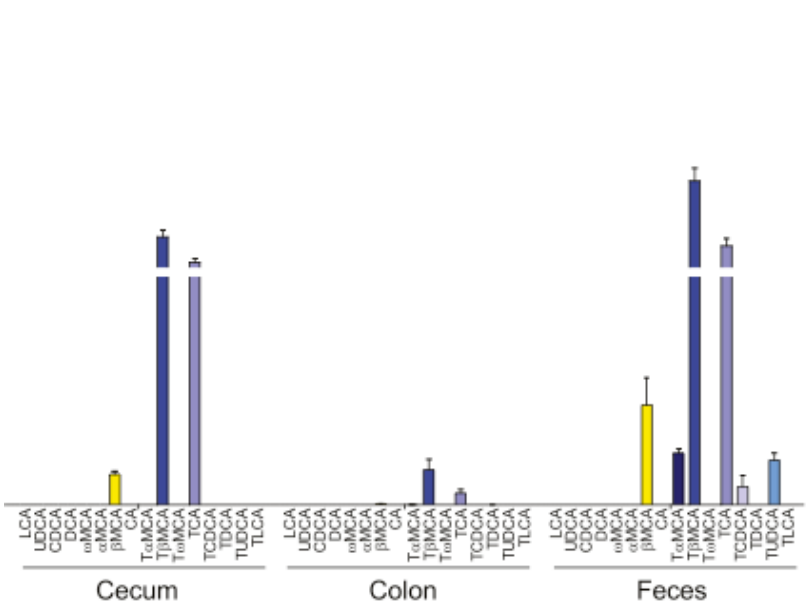
Hofmann, AF (2008)

Bile Acids and Salts

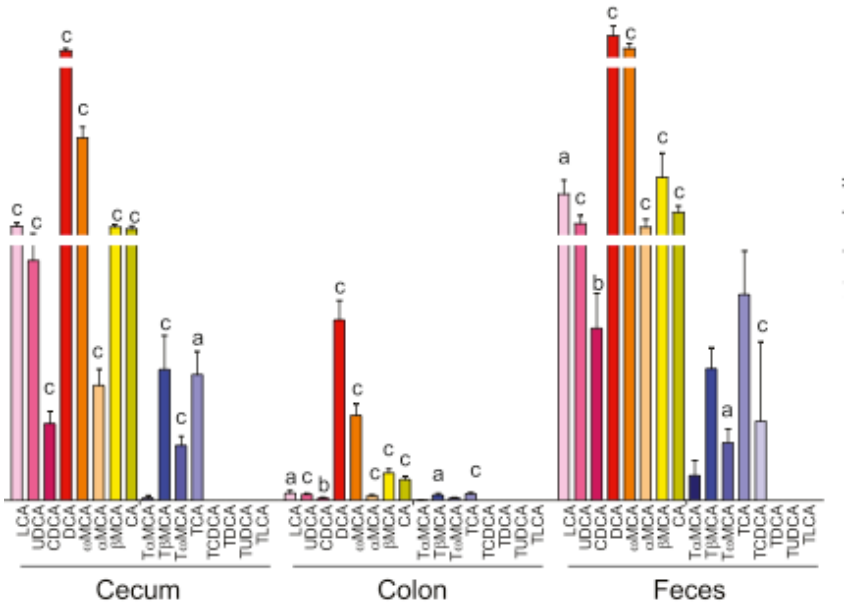


Hofmann, AF (2008)

Microbiota Alter Bile Acid Composition



GF Mice



CONV-R Mice

Sayin et al., *Cell Metab* (2013)

OPEN ACCESS Freely available online

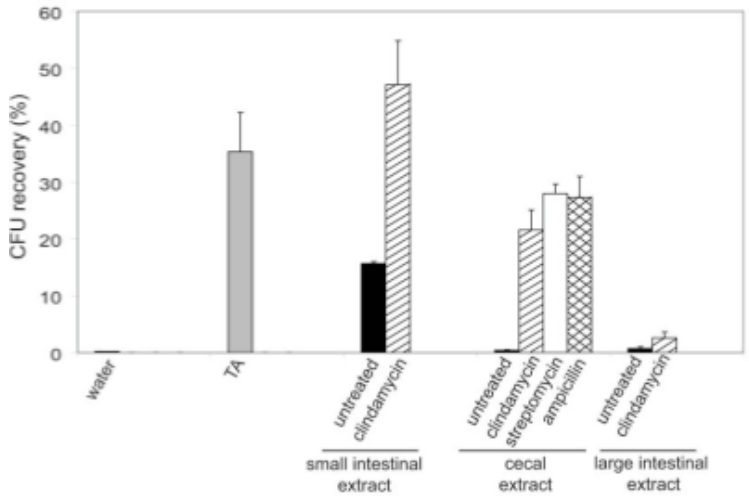
PLoS one

Metabolism of Bile Salts in Mice Influences Spore Germination in *Clostridium difficile*

Jennifer L. Giel[1¤], Joseph A. Sorg[2], Abraham L. Sonenshein[2], Jun Zhu[1*]

1 Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States of America, 2 Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, United States of America

Bile salts in *C. difficile* spore germination



Giel, JL (2010)

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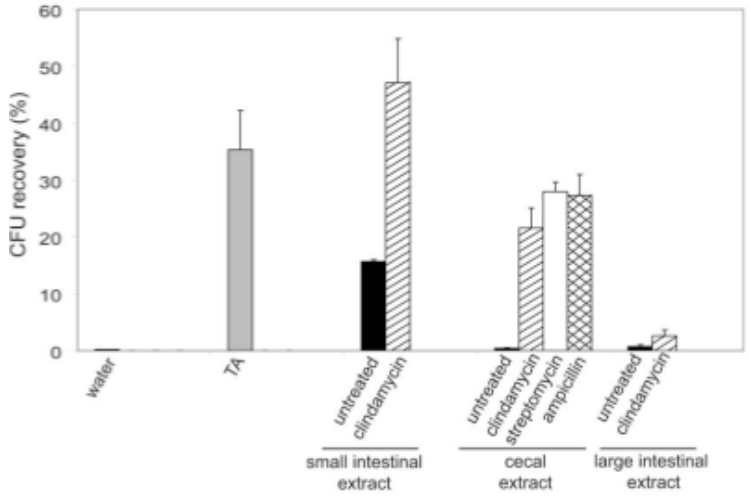

Germination Factor is:

- small
- heat-stable
- water-soluble
- sensitive to cholestyramine

Giel, JL (2010)

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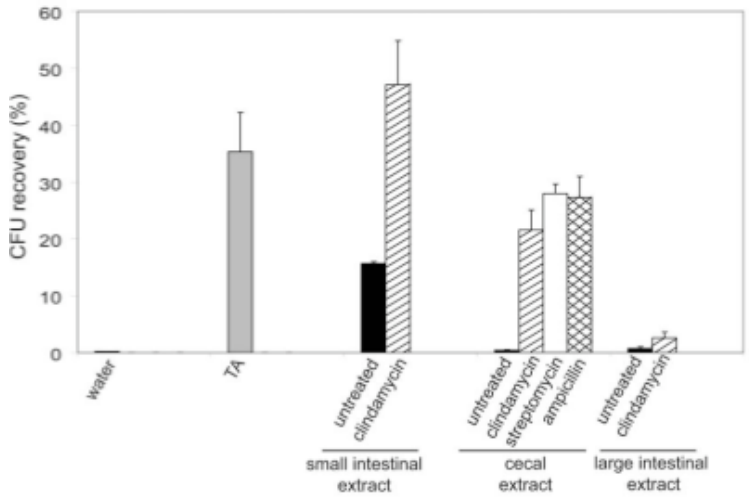

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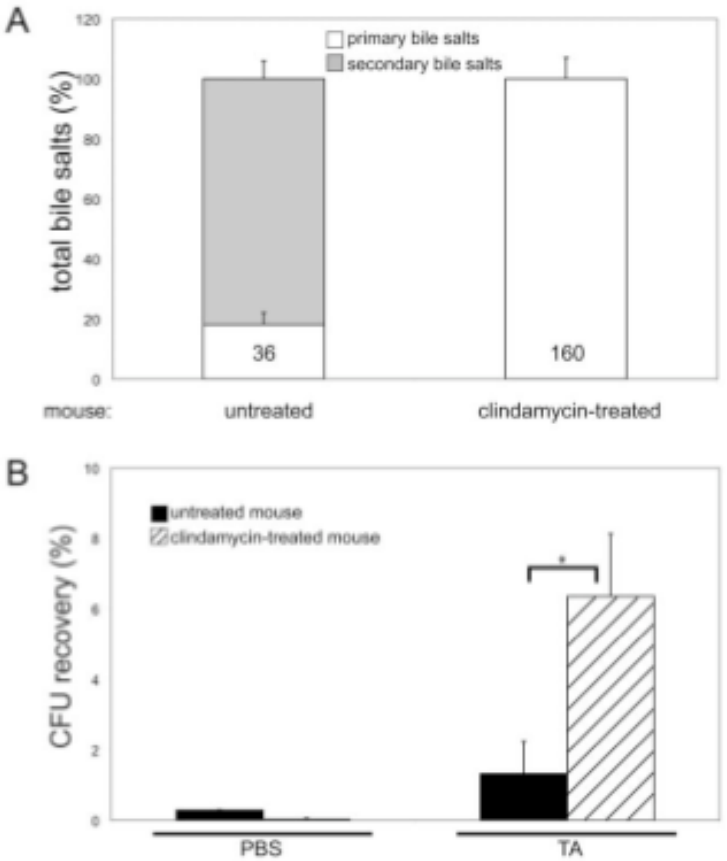
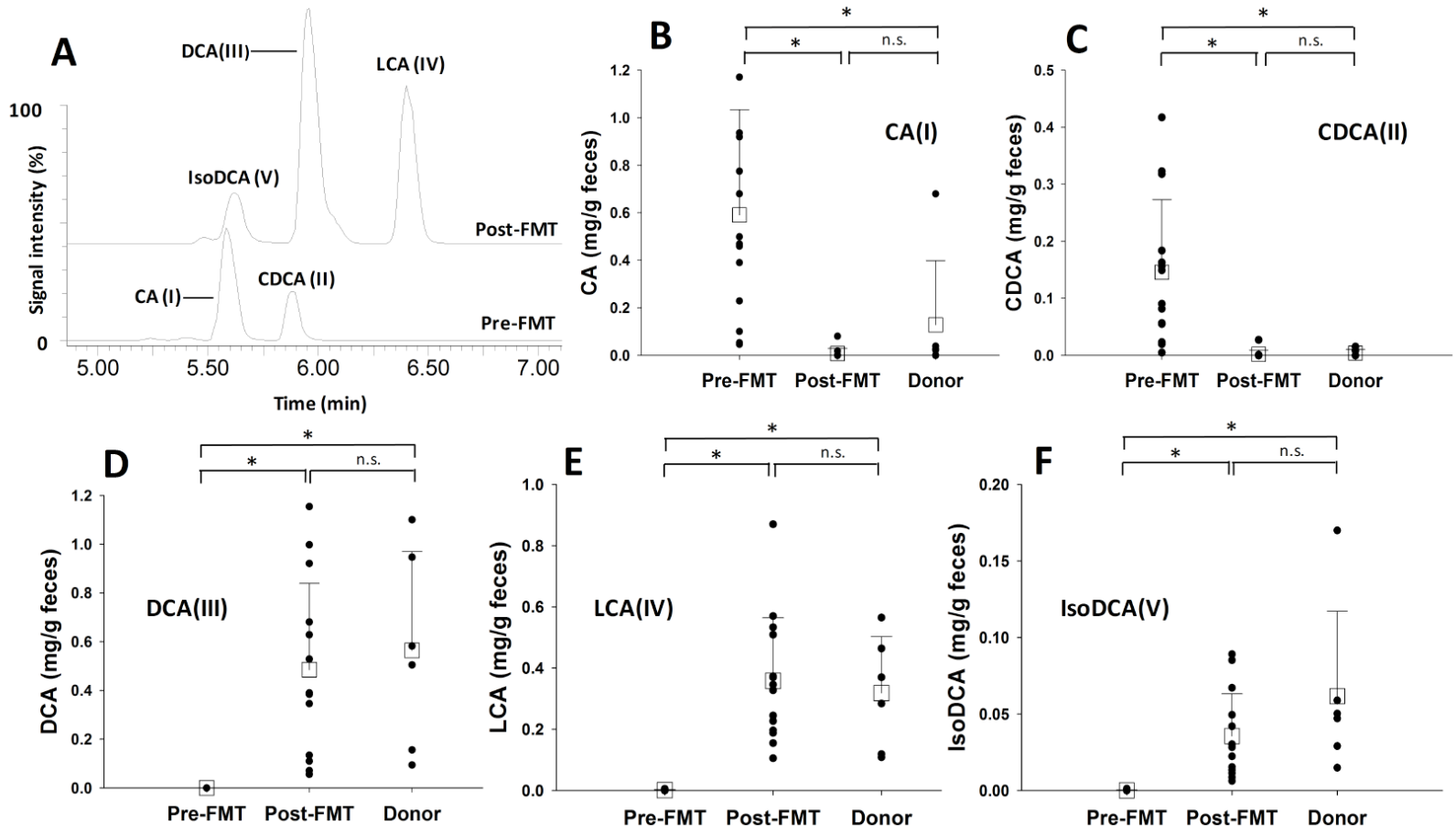


A. Clindamycin eliminates secondary bile salts in feces
B. Fecal bacteria block pro-germinant activity of TA

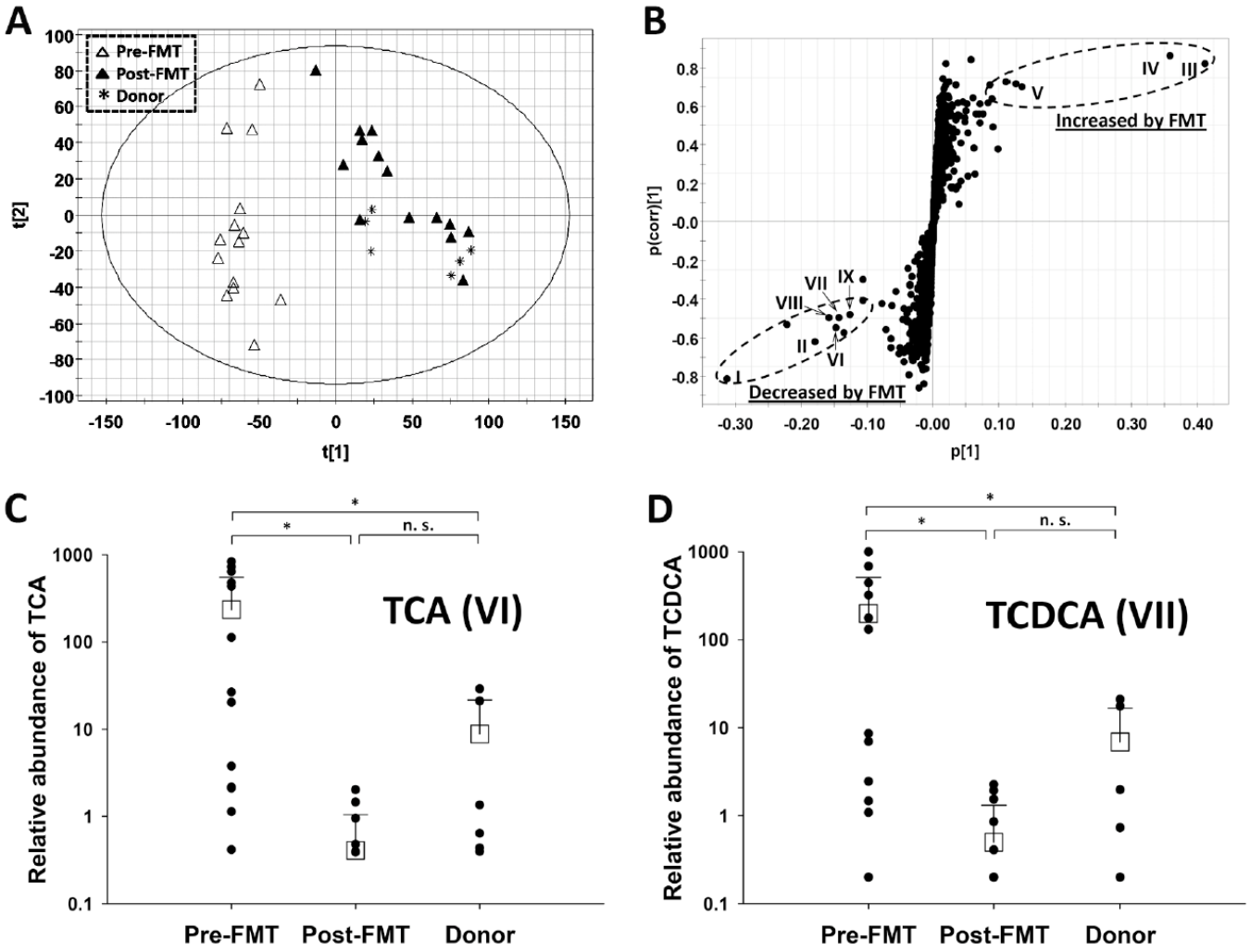
Giel, JL (2010)

Fecal bile acids pre- and post-FMT



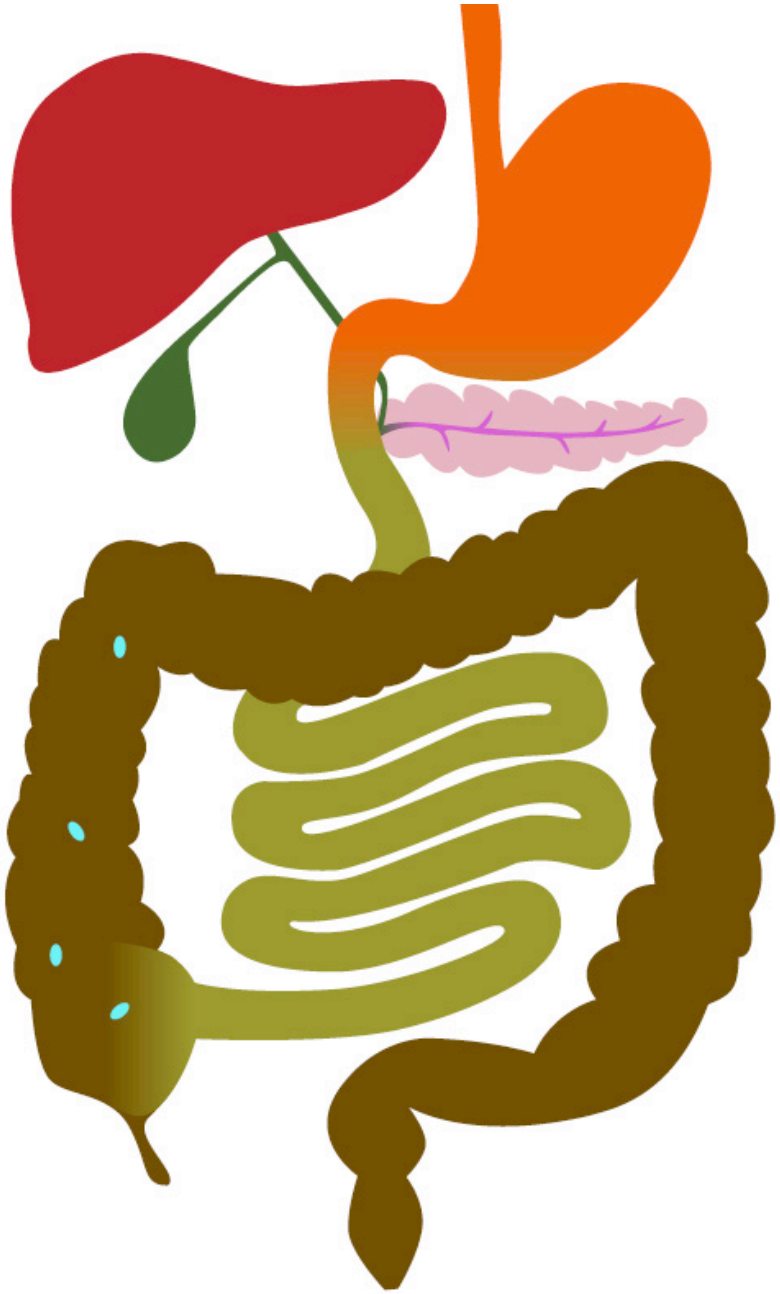
Weingarden et al., Poster #45

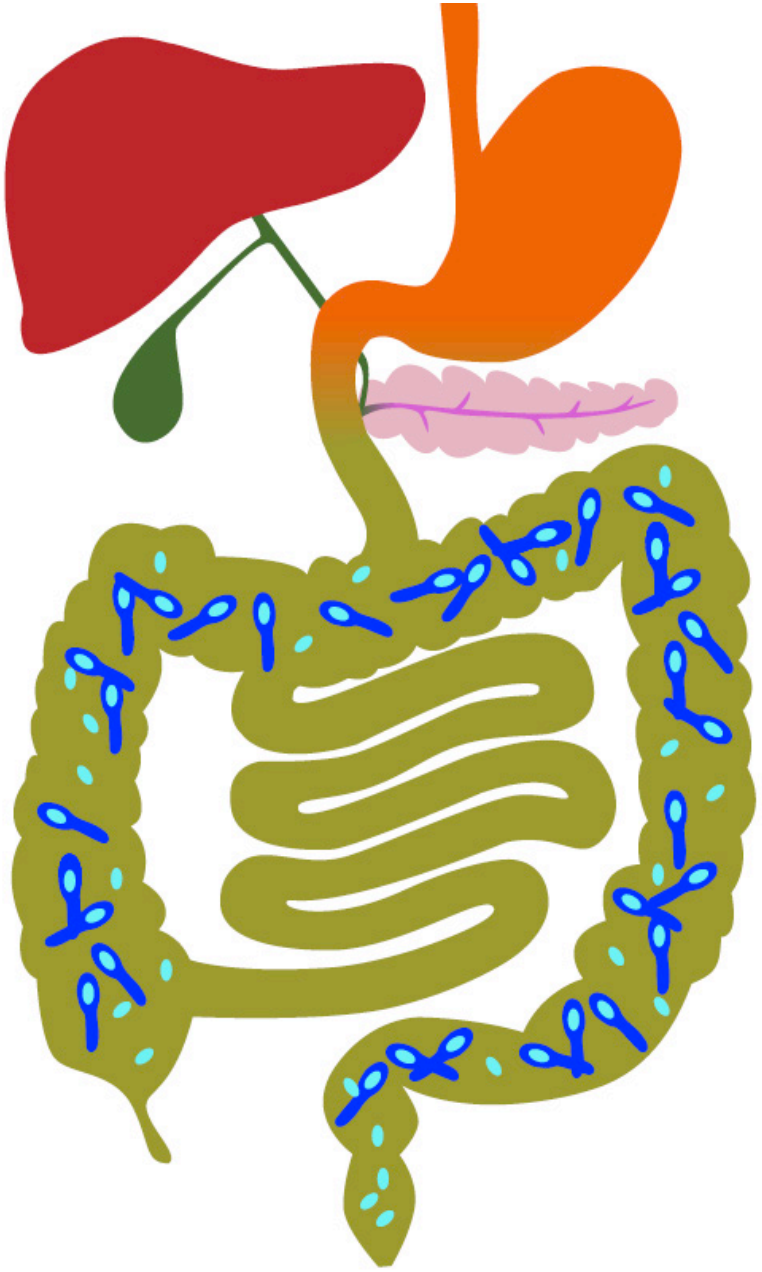
Pre- and post-FMT untargeted metabolomics



Weingarden et al., Poster #45

ID	[M-H]$^-$	Formula	Identity	Effect of FMT
I	407.2798	$C_{24}H_{40}O_5$	cholic acid (CA)	↓
II	391.2848	$C_{24}H_{40}O_4$	chenodeoxycholic acid (CDCA)	↓
III	391.2848	$C_{24}H_{40}O_4$	deoxycholic acid (DCA)	↑
IV	375.2899	$C_{24}H_{40}O_3$	lithocholic acid (LCA)	↑
V	391.2848	$C_{24}H_{40}O_4$	isodeoxycholic acid (isoDCA)	↑
VI	514.2839	$C_{26}H_{45}NO_7S$	taurocholic acid (TCA)	↓
VII	498.2889	$C_{26}H_{45}NO_6S$	taurochenodeoxycholic acid (TCDCA)	↓
VIII	464.2817	$C_{26}H_{43}NO_6$	glycocholic acid (GCA)	↓
IX	448.3063	$C_{26}H_{43}NO_5$	glycoochenodeoxycholic acid (GCDCA)	↓



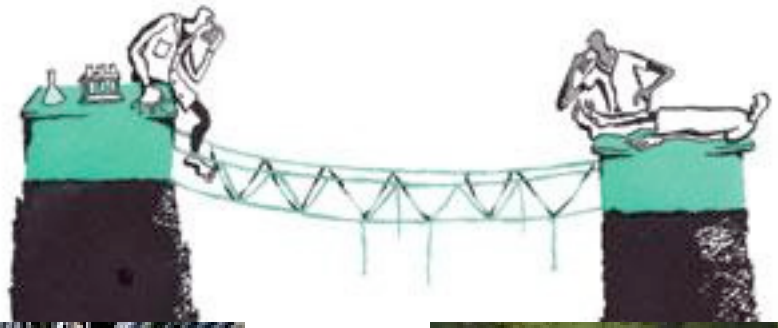


Current Directions Include:

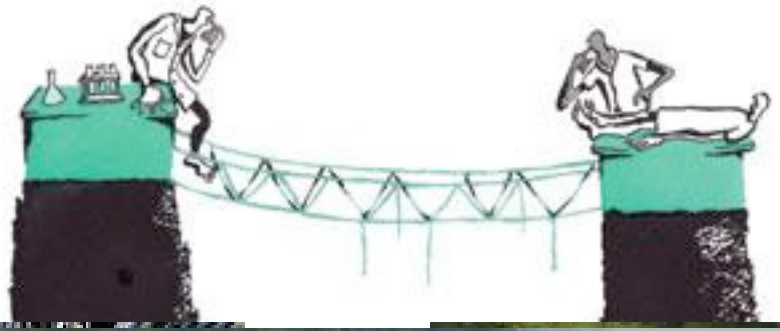
- Further Development of Standardized Full-Spectrum Microbiota for Therapeutic Transplantation
- Expansion of GMP Manufacturing of Full-Spectrum Microbiota
- Mechanism-based Development of Disease Targeted Microbiota Therapeutics

Gaps and Challenges

Gaps and Challenges



Gaps and Challenges



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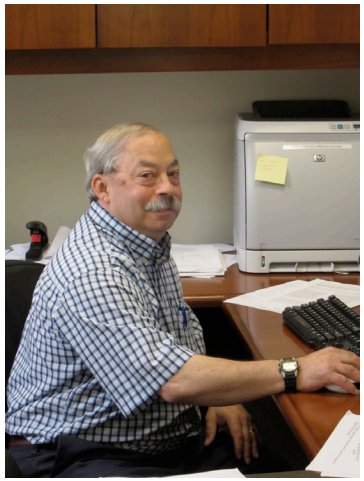

Michael Sadowsky, PhD
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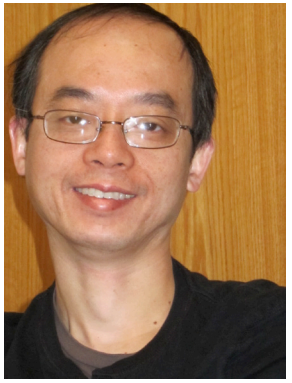
Matthew Hamilton, PhD
Post-Doctoral Fellow

Alexa Weingarden,
MD/PhD Student



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Antonio Gonzalez
Yoshiki Vazquez-Baeza
Neil Lloyd

Wright State University

Oleg Pally

University of Minnesota, Fairview

All nurses (U of M campus, Maple Grove, Riverside Endoscopy)

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