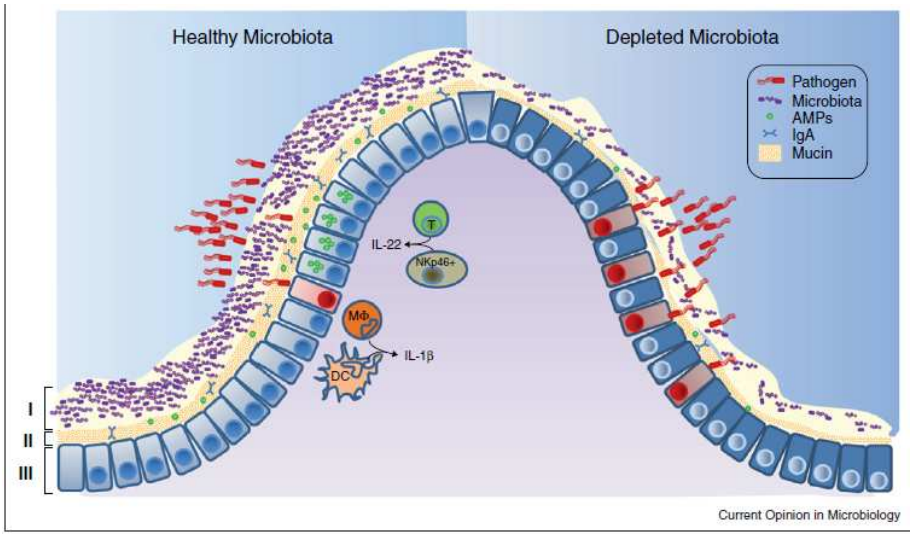
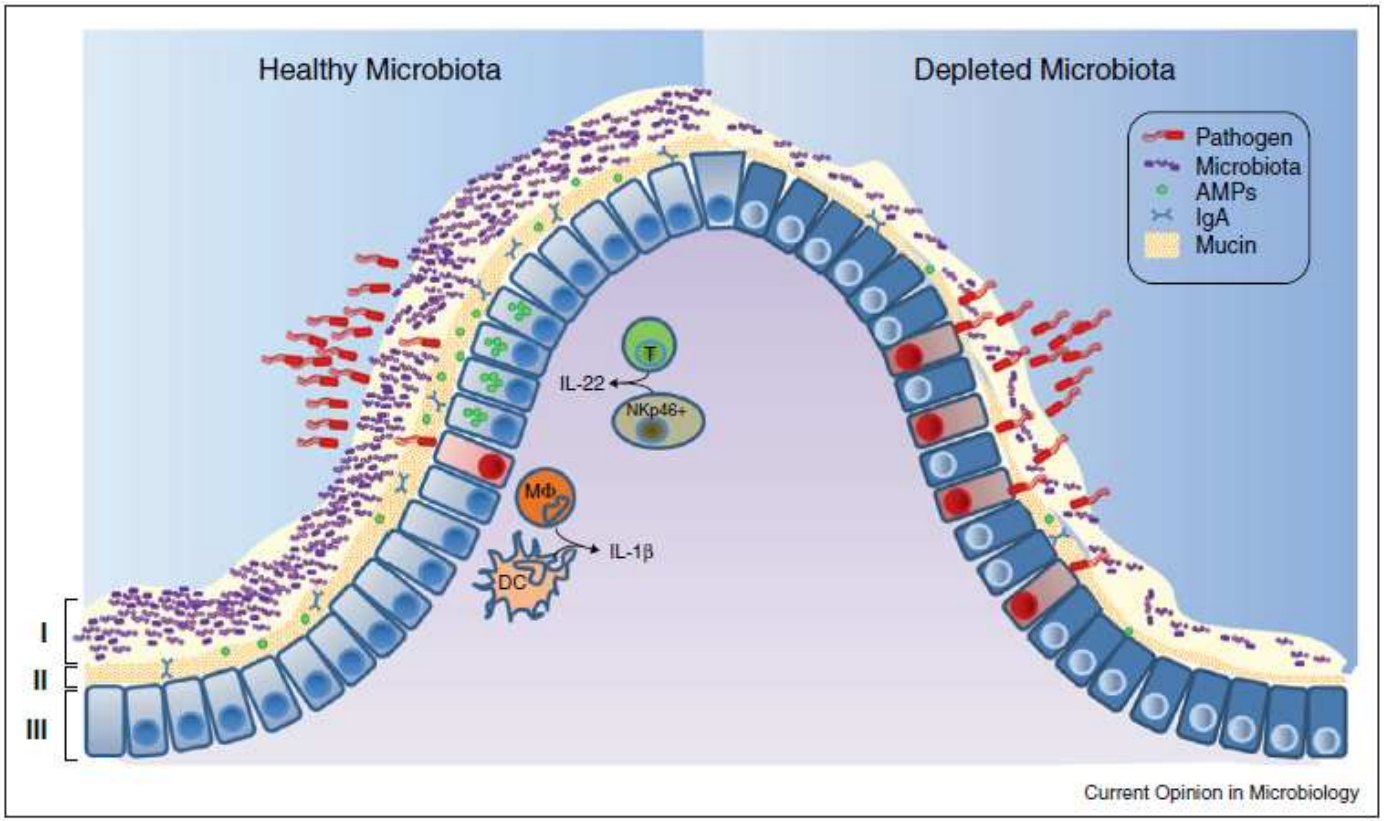
Gut Microbiota and Health





The intestinal microbiota promotes three levels of protection against enteric infection. **(I)** Saturation of colonization sites and competition for nutrients by the microbiota limit pathogen association with host tissue. **(II)** Commensal microbes prime barrier immunity by driving expression of mucin, immunoglobulin A (IgA) and antimicrobial peptides (AMPs) that further prevents pathogen contact with host mucosa. **(III)** Finally, the microbiota enhances immune responses to invading pathogens. This is achieve by promoting IL-22 expression by T cells and NKp46+ cells, which increases epithelial resistance against infection, as well as priming secretion of IL-1β by intestinal monocytes (MΦ) and dendritic cells (DCs), which promotes recruitment of inflammatory cells into the site of infection. In conditions in which the microbiota is absent, such as following antibiotic treatment, there is reduced competition, barrier resistance and immune defense against pathogen invasion.

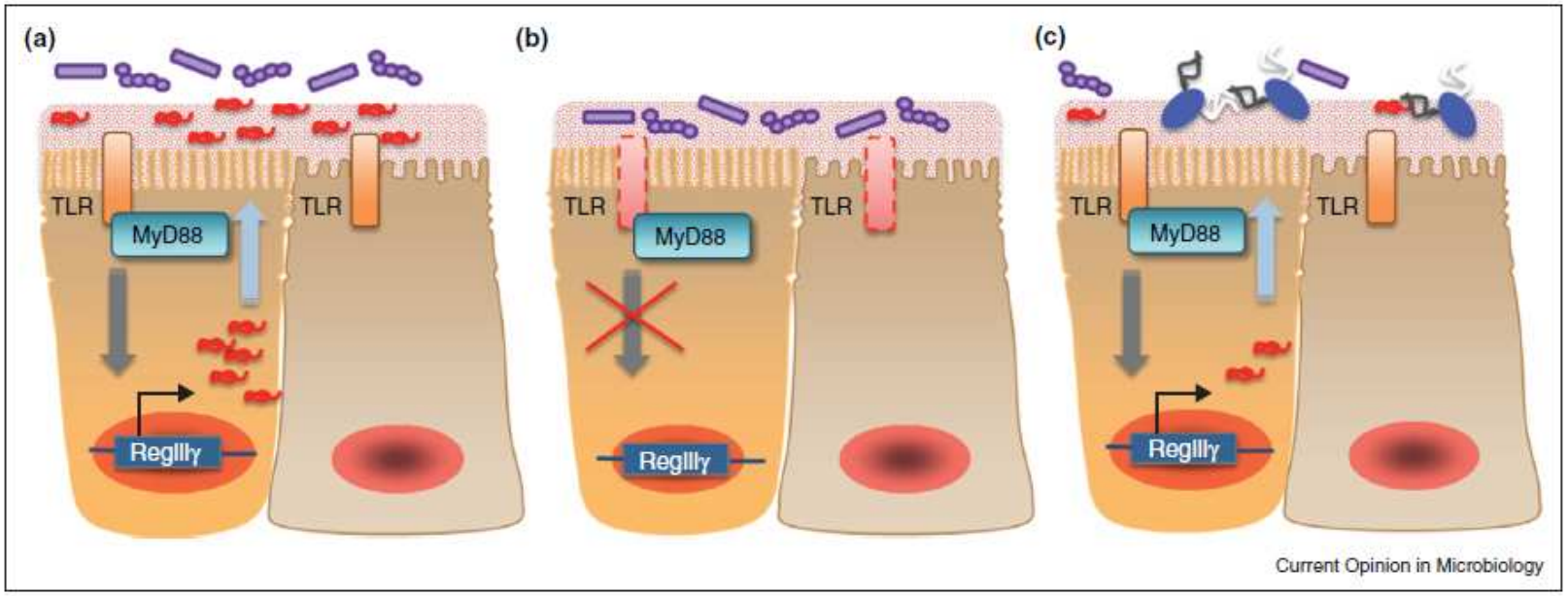
The discovery of antibiotics in the last century is one of the most significant achievements of modern medicine. Pathogens that once devastated entire civilizations, such as *Mycobacterium tuberculosis*, could finally be controlled, suggesting a triumph over infectious disease. However, the rampant rise of antibiotic resistance among pathogens, compounded by a drying pipeline of novel antibiotic development by pharmaceutical companies, has rendered current therapeutic strategies ineffective. As such, it is speculated that we are entering a post-antibiotic era where pathogens once again reign with limited opposition and a minor scrape may pose the risk of a fatal infection [1,2]. To combat the renewed threat of pathogenic microorganisms, clinical approaches toward eradicating infectious disease must evolve.

… certain enteric pathogens are able to outcompete commensal microbes by actively triggering host inflammation which favors pathogen invasion and dissemination.

C. rodentium, *Campylobacter jejuni*, and *Salmonella enterica* serovar Typhimurium (STm) appear to induce inflammation as part of their infectious process, and increasing intestinal inflammation actually promotes disease.

Further, these reports surprisingly demonstrate that **pathogen-induced inflammation** adversely affects the microbiota, **reducing the numbers of beneficial bacteria**, which protect us from infections.

Collectively, there is growing evidence for the notion that pathogens and symbiotic bacteria are engaged in an 'evolutionary combat', with the host serving as the battlefield.



The commensal microbiota primes barrier immunity. Direct stimulation of epithelial Toll-like receptors (TLRs) by commensal MAMPs primes expression of RegIIIγ **(a)**. Production of RegIIIγ is essential to limit microbial contact with host mucosa. As such, defects in TLR function results in deficient RegIIIγ expression resulting in an increased association of commensal microbes with host tissue as well as a heighten risk of infection with enteric pathogens **(b)**. Additionally, reduced TLR stimulation as a consequence of the depletion of the microbiota is sufficient to reduce RegIIIγ expression and render the host susceptible to infection (c).

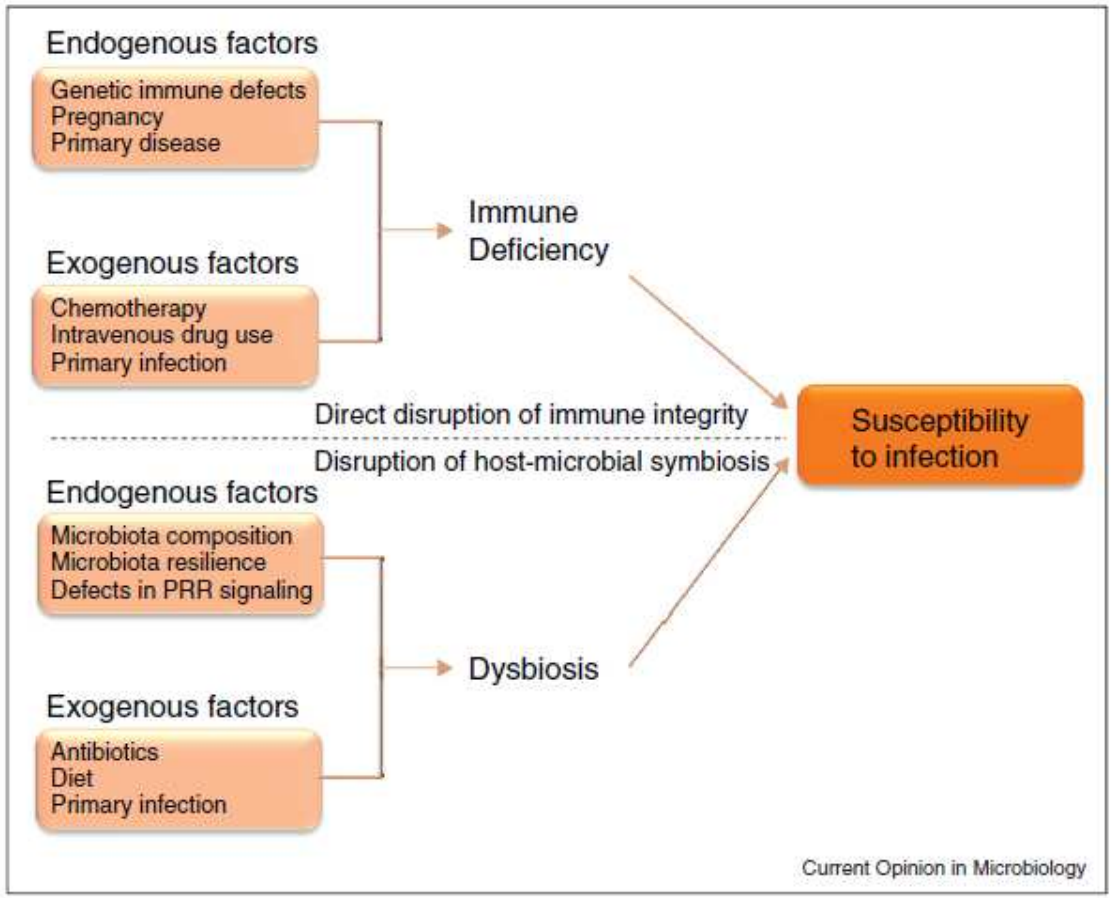
RegIIIg
is a C-type lectin that possesses antimicrobial activity against Gram-positive microbes.

MAMPs
icrobial associated molecular patterns

TLRs
pathogen recognition receptors

Defects in host–microbial symbiosis may predicate susceptibility to infection

… factors that determine an individual's susceptibility to infectious disease remain largely unknown.



PRR
pathogen recognition receptors

The evidence summarized in this review suggests that **disruption of the microbiota through environmental influences may compromise immune function, leading to increased susceptibility to infectious disease**.

In particular, we propose that antibiotic use **may paradoxically promote bacterial and viral infections** by depleting immune-promoting gut bacteria.

For example, antibiotics are routinely administered in the hospital to patients admitted for various non-bacterial illnesses. Not only can this practice select for antibiotic-resistant microbes (an extensively reported phenomenon), but may also lead to nosocomial infections by reducing the ability of the immune system to fight infections.

Furthermore, antibiotic use over several generations may reduce gut bacteria diversity in entire populations, a notion proposed by the 'disappearing microbiota' hypothesis. In cases where antimicrobial use is justified, we speculate that the administration of commensal-derived products that promote immunity may represent a viable companion therapy to antibiotics … **efforts that support microbiome-mediated protection may be an effective approach to achieve resistance to infectious disease in the postantibiotic era**.

Bacteriotherapy for the treatment of intestinal dysbiosis caused by *Clostridium difficile* infection

Faecal microbiota transplantation (**FMT**) has been used for more than five decades to treat a variety of intestinal diseases associated with pathological imbalances within the resident microbiota, termed dysbiosis.

FMT has been particularly effective for treating patients with recurrent ***Clostridium difficile*** infection who are left with few clinical options other than continued antibiotic therapy.

Our increasing knowledge of the structure and function of the human intestinal microbiota and *C. difficile* pathogenesis has led to the understanding that **FMT promotes intestinal ecological restoration** and **highlights the microbiota as a viable therapeutic target**.

However, the use of undefined faecal samples creates a barrier for widespread clinical use because of safety and aesthetic issues.

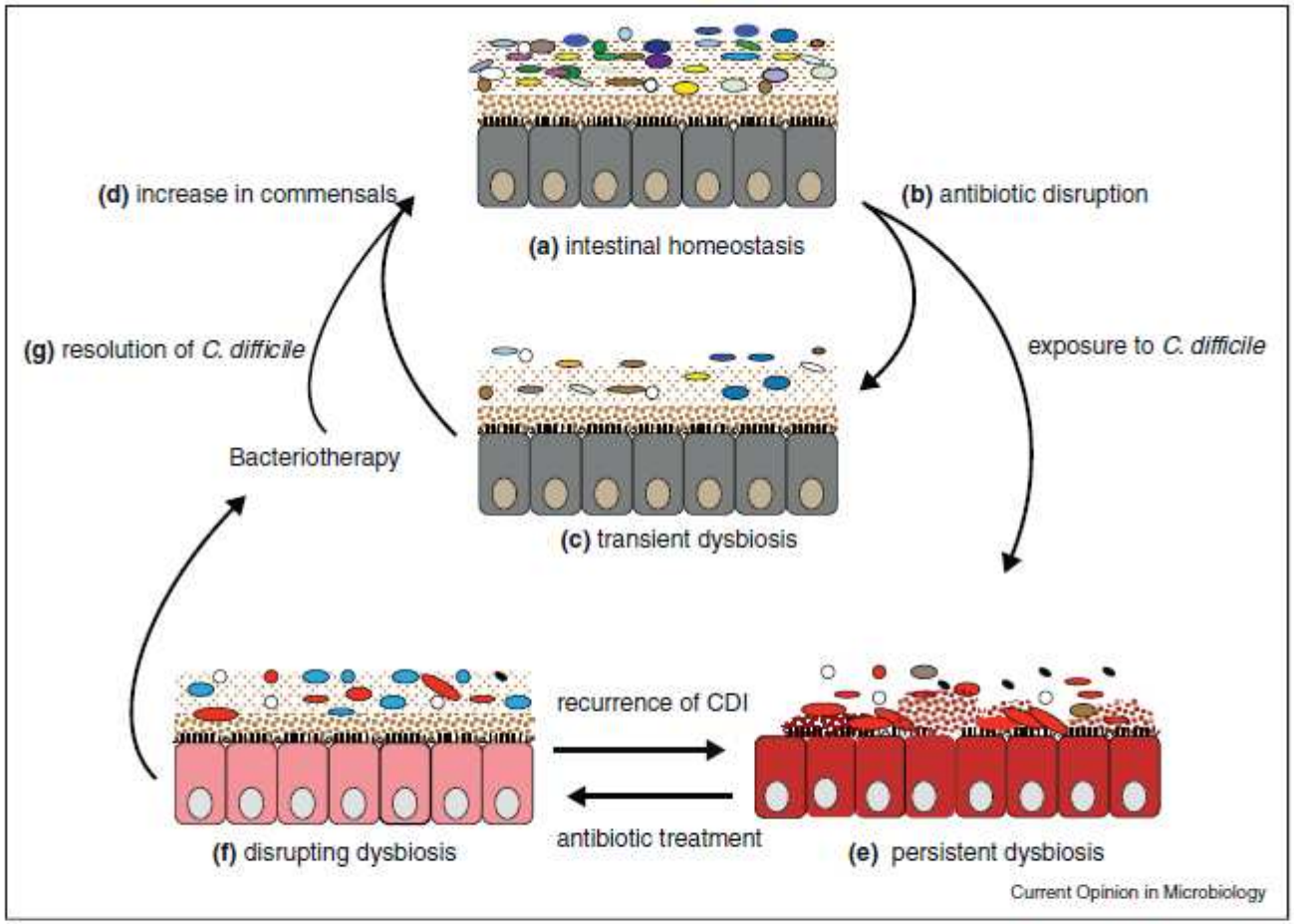
An emerging concept of bacteriotherapy, the therapeutic use of a **defined mixture of harmless, health-associated bacteria**, holds promise for the treatment of patients with severe *C. difficile* infection, and possibly represents a paradigm shift for the treatment of diseases linked to intestinal dysbiosis

Disturbances in the intestinal microbiota caused by infections and antibiotics have profound effect on the microbiota's composition and function and can predispose the individual to **antibiotic-associated diar-rhoea (AAD)**. In the last decade, the incidence of mor-bidity and mortality from *Clostridium difficile* infection (**CDI**), the leading cause of **AAD**, increased largely due to the emergence and **global spread of fluoroquinolone- resistant variant** of ***C. difficile***.

First line **treatment** for severe *C. difficile* infections include metronidazole or vancomycin although in **15–35% of these cases, a recurrence** (**relapse or reinfection**) **follows the cessation of antibiotic therapy**.

FMT has been mainly used as an alternative treatment for patients with persistent, recurrent *C. difficile* infection and involves the restoration of the intestinal microbiota through instillation of homogenized faecal suspension from a healthy donor.

***FMT** has also been utilized in the treatment of diseases associated with intestinal dysbiosis such as inflammatory bowel diseases (IBD; manifested as Crohn's disease and ulcerative colitis), irritable bowel syndrome (IBS) and obesity.*



A proposed model for recurrent *C. difficile* infection and the restoration of the intestinal microbiota through FMT or bacteriotherapy. Intestinal homeostasis **(a)** is characterized by large microbial diversity in the microbiota and health-associated metabolites. Antibiotic exposure disrupts the microbiota **(b–c)** by destroying the microbial community leading to reduction in the diversity and loss of colonization resistance. The microbiota usually expands in diversity **(d)** after antibiotics are stopped to restore diversity. Antibiotic disruption makes individuals hyper-susceptible to *C. difficile* colonization potentially leading to chronic infection and persistent dysbiosis **(e)**. After treatment of CDI with antibiotics such as vancomycin, further microbiota disruption **(f)** occurs potentially leading to recurrent CDI after discontinued use of the antibiotic. FMT or bacteriotherapy disrupts intestinal dysbiosis leading to resolution of CDI **(g)** and increase in species diversity (d) and restores intestinal homeostasis.
Figure modified from [48].

C. difficile produces highly resistant and transmissible **spores** that can potentially persist in the gut of infected individuals or contaminate skin or environmental surfaces.

These spores are resistant to antibiotics and can recolonize and germinate after antibiotic therapy leading to recurrent disease. Asymptomatic carriers are also a source of spores that can promote transmission and persistence at both the hospital and global levels.

…**patients with recurrent CDI had decreased proportional abundance of Bacteroidetes and increased Proteobacteria and Verrucomicrobia.**

*.. the use of **new antibiotic**, fidaxomicin was found to have lower rates of recurrence of **C. difficile** infection associated with the non-epidemic strain compared to vancomycin, an effect that is attributed to its **lower activity against commensal and beneficial gut microbes***

FMT

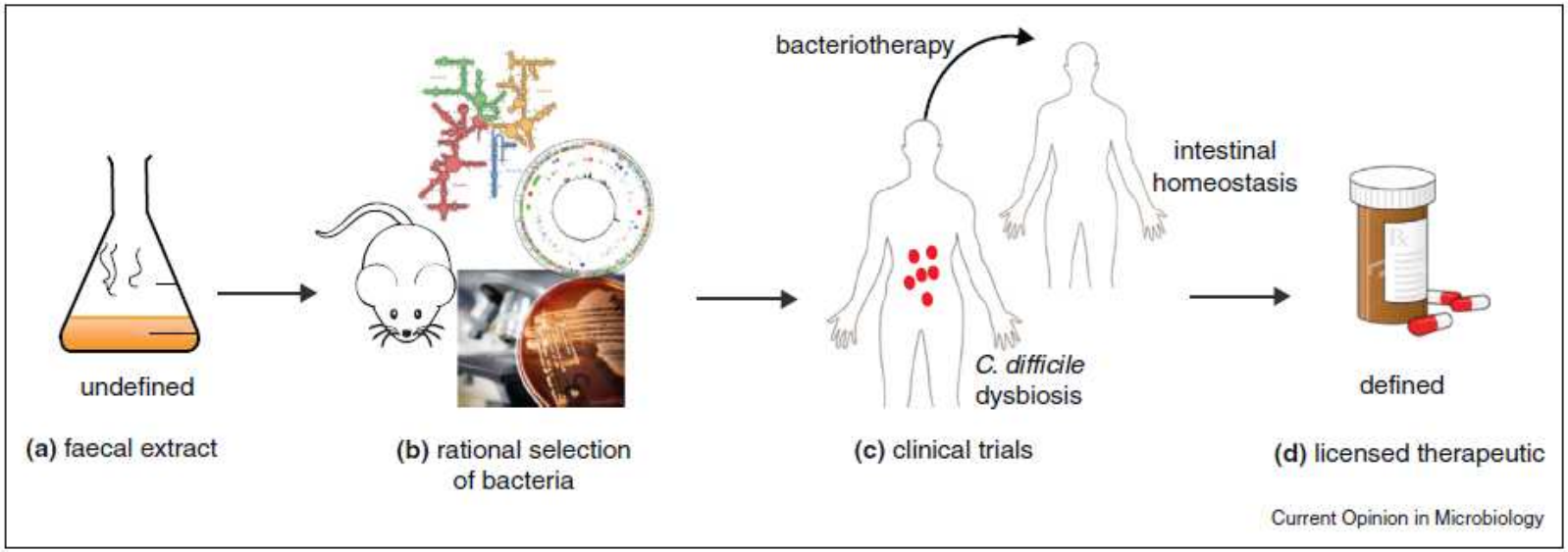
In this process, homogenized faeces from a healthy donor is infused through colonoscopy, enema or nasogastrically to an individual with *C. difficile* disease to restore the intestinal microbiota and thereby eradicate CDI. **The donor, usually a healthy individual/relative, is screened for contagious pathogenic agents such as *Salmonella* spp., *Staphylococcus aureus*, *C. difficile* and HIV and other infections or inflammatory conditions.**

The intestinal microbiota of *C. difficile* patients treated with **FMT** is characterized by expansions in species diversity characterized by an increase in *Bacteroides*, *Roseburia* and ***Faecalibacterium*** and a reduction in *Enterobacteriaceae.*

Rational design of a defined bacteriotherapy

The use of undefined faecal samples for FMT creates a barrier for widespread clinical use, mainly because of the **amount of time needed** to prepare and **screen donor samples**, **patient safety issues**, **non-standardization** of the treatment procedure and general doctor and patient **aversion**.

Therefore, there is an unmet clinical need **to design a combination of harmless, health associated bac- teria as a viable therapeutic option.**



Generic model to create a standardized, defined bacteriotherapy mixture for treatment of patients with severe CDI. Culturing and genomic profiling of faecal samples from healthy donors and CDI patients **(a)** could potentially identify candidate bacteria that can be tested *in vivo* for safety and efficacy. Whole genome sequencing will define the bacteria and provide a basis to determine phylogenetic position within the microbiota community **(b)**. Clinical trials **(c)** will be required to test efficacy of bacteriotherapy mixture in diseased humans with severe CDI before widespread clinical use **(d)**.

FMT is increasingly being accepted as a treatment for recurrent **CDI**, but large, randomized double-blinded studies are needed. However, beyond FMT, **bacteriotherapy using standardized mixtures** of beneficial bacteria could potentially be used in the future for the treatment of recurrent **CDI** and other diseases associated with dysbiosis in the intestinal microbiota such as **IBD**, **IBS** and **obesity**

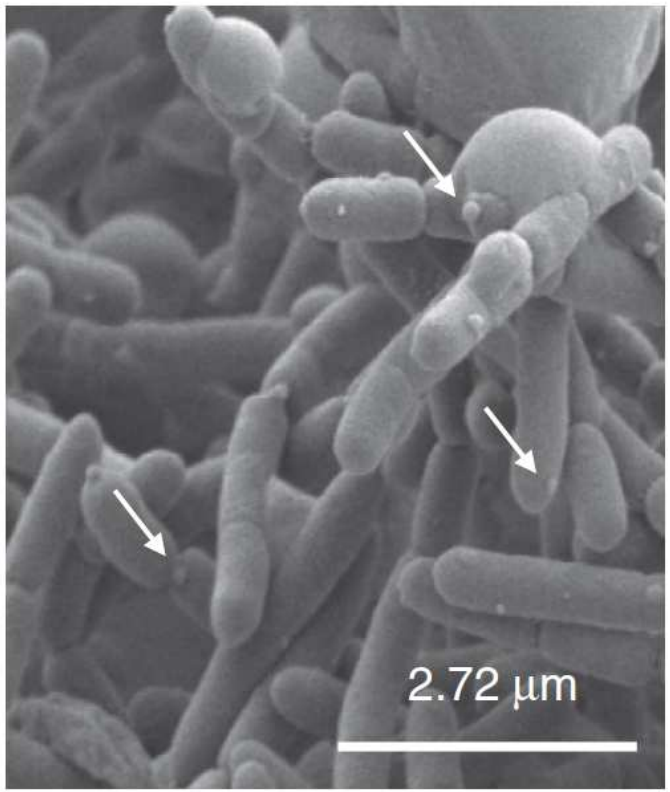
Faecalibacterium prausnitzii and human intestinal health

Faecalibacterium prausnitzii is the most abundant bacterium in the human intestinal microbiota of healthy adults, representing more than 5% of the total bacterial population.

Over the past five years, an increasing number of studies have clearly described the importance of this highly metabolically active commensal bacterium as a component of the healthy human microbiota.

Changes in the abundance of *F. prausnitzii* have been linked to dysbiosis in several human disorders.

Administration of *F. prausnitzii* strain A2-165 and its culture supernatant have been shown to protect against 2,4,6- trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice

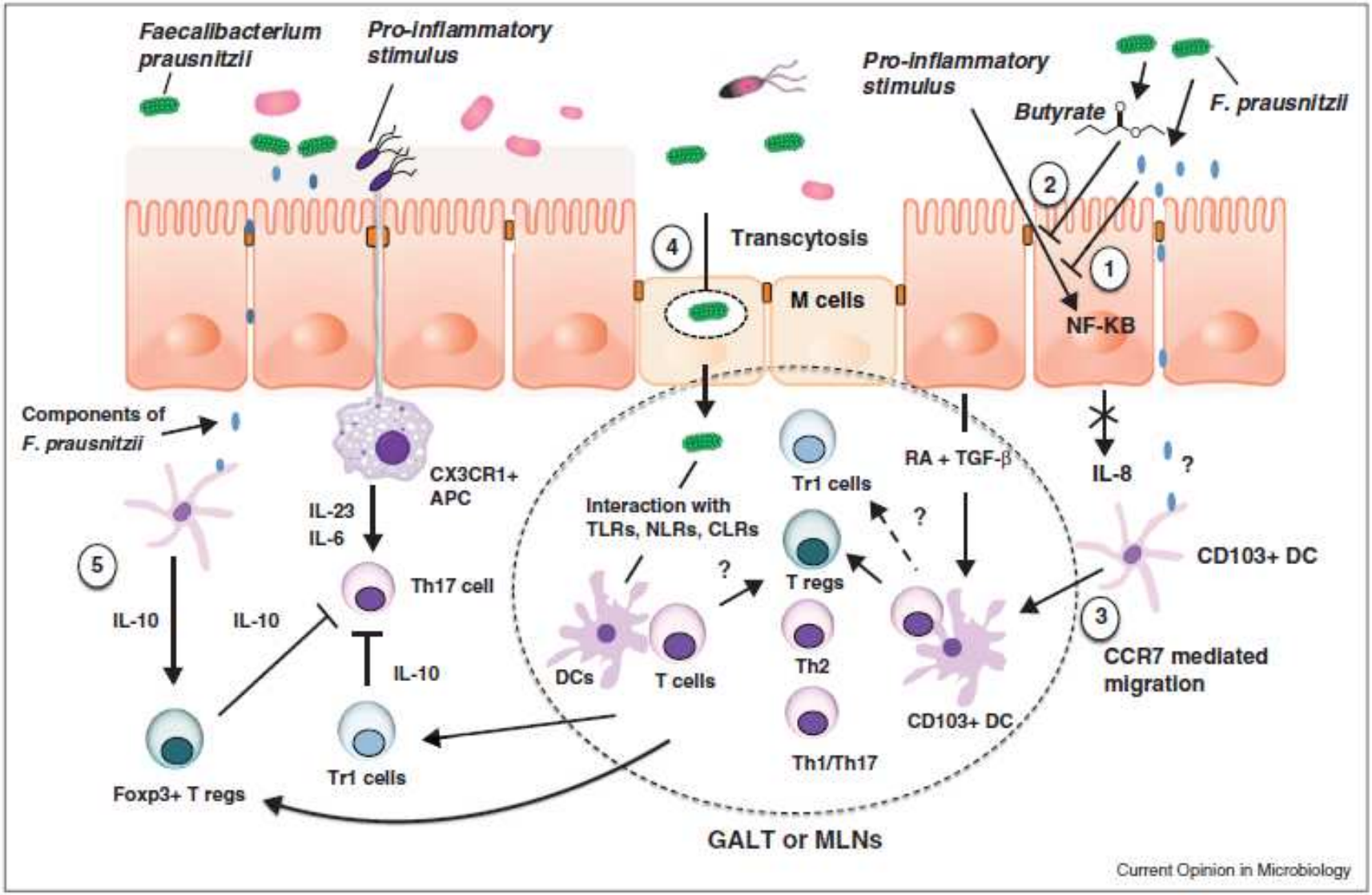


***F. prausnitzii* variations in the microbiota of different IBD cohorts compared to healthy subjects**

Diseases	Samples	Techniques	*n*	Mean ages	*F. prausnitzii* variations
UC	Colonic	Sequencing 16S rRNA	1	12	↑
UC	Fecal	FISH	105	41.2 (18–84)	↑
UC	Fecal	PCR	14	ND	→
UC	Colonic biopsy	Pyrosequencing/RT-PCR	12	13.0 (8.5–15.8)	→
R-UC	Fecal	RT-qPCR	4	35 (±4.3)	→
UC	Mucosal pouch biopsy	Sequencing 16S rRNA	8	51 (19–63)	↓
UC	Fecal	*In vitro*: M-SHIME	6	40.5 (33–78)	↓
UC	Fecal	Real time PCR	22	38.4 (±11.3)	↓
A-UC	Fecal	RT-qPCR	13	39.7 (±3.5)	↓
Pouchitis	Mocosal biopsy	Sequencing 16S rRNA	8	39 (19–64)	→
Pouchitis FAP	Mocosal biopsy	Sequencing 16S rRNA	3	32 (30–54)	→
CD	Colonic biopsy	Pyrosequencing/RT-PCR	13	12.2 (8.0–16.3)	↑
CD	Biopsy	PCR-DGGE	19	36.7 (±3.72)	↓
CD	Fecal	FISH	82	34.8 (17–78)	↓
CD	Fecal	PCR	20	ND	↓
CD	Fecal	RT-qPCR/microarray	16	31(25–39)	↓
CD	Fecal	FISH	50	39 (19–68)	↓
CD	Fecal	FISH	28	44.3 (21–76)	↓
CD	Fecal	DGGE	68	45 (25–76)	↓
CD	Fecal	RT-qPCR	47	35.3 (±9.4)	↓
CD	Fecal	Real time PCR	20	31.2 (±14.1)	↓
RCD	Fecal	RT-qPCR	10	39.1 (±4.2)	→
RCD	Fecal	GoArray	6	31 (18–44)	↓
ACD	Fecal	RT-qPCR	22	36.9 (±3.3)	↓
ACD	Mucosa associated	FISH	ND	ND	↓
ACD	Fecal	FISH	103	ND	↓
ICD	Mucosa associated	RT-qPCR	6	50.8 (±4.5)	↓
ICD	Ileal biopsy	qPCR	18	35.2 (18–58)	↓
CCD	Mucosa associated	RT-qPCR	8	49 (±18.5)	→

UC, ulcerative colitis; AUC, active-ulcerative colitis; RUC, remission-ulcerative colitis; FAP, familial adenomatous polyposis; CD, Crohn's
ACD, active Crohn's disease; RCD, remission Crohn's disease; CCD, colonic Crohn's disease; ICD, ileal Crohn's disease; ND, not dete

Correlation have been found between the level of *F. prausnitzii* and obesity and Coeliac disease.



Proposed anti-inflammatory mechanisms of *F. prausnitzii*. 1. The supernatant of *F. prausnitzii* blocks NF-κB activation induced by a pro-inflammatory stimulus [21••]. 2. Butyrate produced by *F. prausnitzii* inhibits NF-κB activation in mucosal biopsies. 3. *F. prausnitzii* components might interact with CD103+ dendritic cells (DCs) in the lamina propria and stimulate their migration to mesenteric lymph nodes (MLN) and the induction of Tregs. 4. M cell transcytosis of *F. prausnitzii* in organized lymphoid structures may induce Tregs. 5. The capacity of *F. prausnitzii* to induce high amounts of IL-10 in antigen presenting cells may enhance the suppressive activity of Foxp3+ Tregs and block Th17 cells induced by pro-inflammatory stimuli.

F. prausnitzii, is a major EOS (extremely oxygen sensitive) component of the intestinal microbiota which has been largely ignored until recently. Its low prevalence in many intestinal disorders, particularly in IBD patients, suggests its **potential as an indicator of intestinal health**.

F. prausnitzii **is a butyrate producer** and has demonstrated antiinflammatory effects in vitro and in vivo using a mouse colitis model making it a key member of the microbiota that may contribute to intestinal homeostasis.
Thus, modulation of ***F. prausnitzii*** abundance, for example using prebiotics and/or probiotics and/or formulations that permit survival through the upper part of the intestinal tract might have prophylactic or therapeutic applications in human health.

For instance, the fiber inulin has well-characterized impact on microbiota composition inducing specific and significant increase in *Bifidobacterium* and *F. prausnitzii*.

Moreover the rapid detection of ***F. prausnitzii*** abundance in feces warrants further investigation as a biomarker of intestinal health.

Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice

READ THE FULL ARTICLE ONLINE
http://dx.doi.org/10.1126/science.1241214
Cite this article as V. K. Ridaura *et al.*, *Science* **341**, 1241214 (2013).
DOI: 10.1126/science.1241214

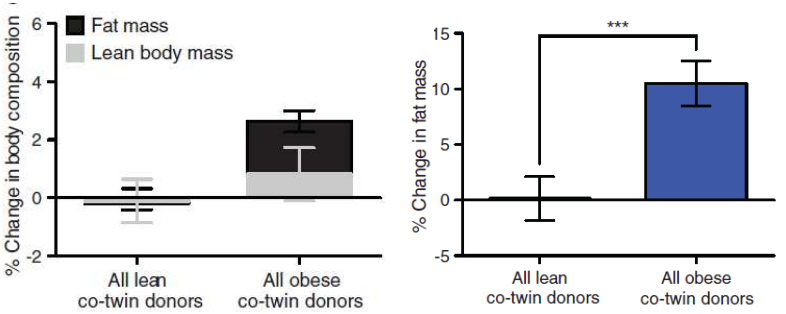
We **transplanted fecal microbiota** from **adult female twin pairs discordant for obesity** into **germ-free mice** fed low-fat mouse chow, as well as diets representing different levels of saturated fat and fruit and vegetable consumption typical of the U.S. diet.

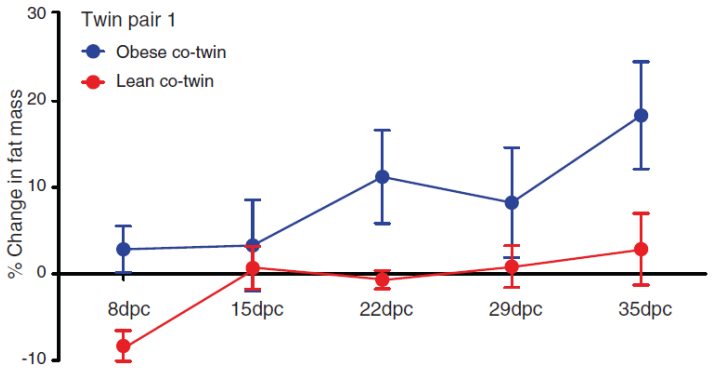
Increased total body and fat mass, as well as obesity-associated metabolic phenotypes, were transmissible with uncultured fecal communities and with their corresponding fecal bacterial culture collections.

Cohousing mice harboring an obese twin's microbiota (Ob) with mice containing the lean co-twin's microbiota (Ln) prevented the development of increased body mass and obesity-associated metabolic phenotypes in Ob cage mates … was diet-dependent.

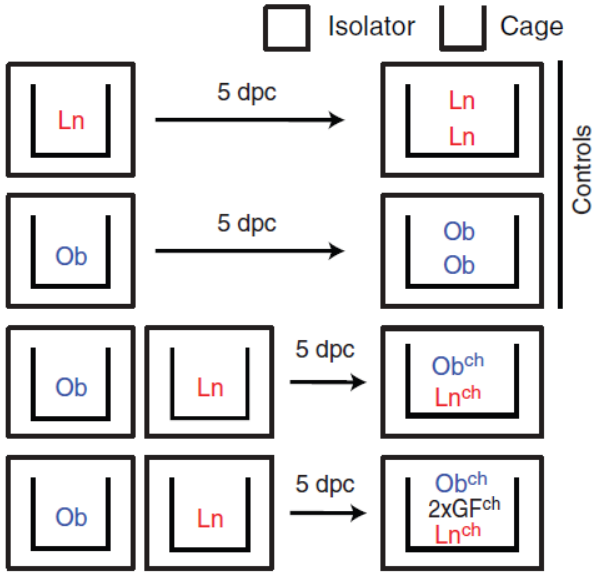
Monozygotic (MZ) or dizygotic (DZ) twins discordant for obesity provide an attractive model for studying the interrelations between obesity, its associated dietary and lifestyle risk factors, and the gut microbiota/microbiome. **In the case of same-sex twins discordant for a disease phenotype, the healthy co-twin provides a valuable reference control to contrast with the co-twin's disease associated gut community.**

Twin Pair	1 (DZ)		2 (DZ)		3 (DZ)		4 (MZ)	
BMI (kg/m^2)	23	32	25.5	31	19.5	30.7	24	33

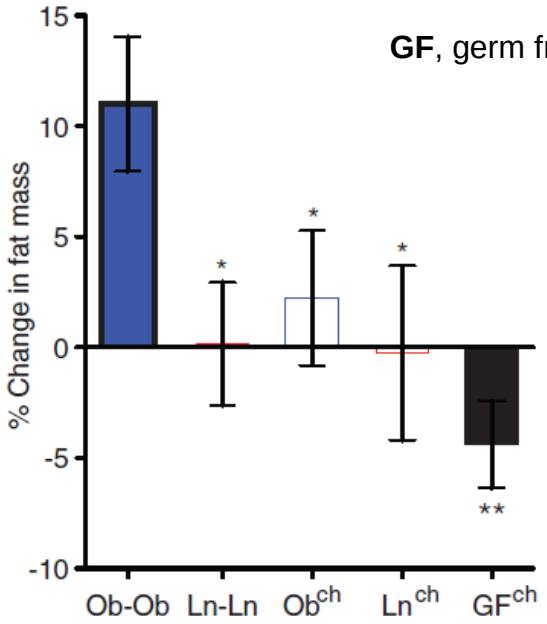


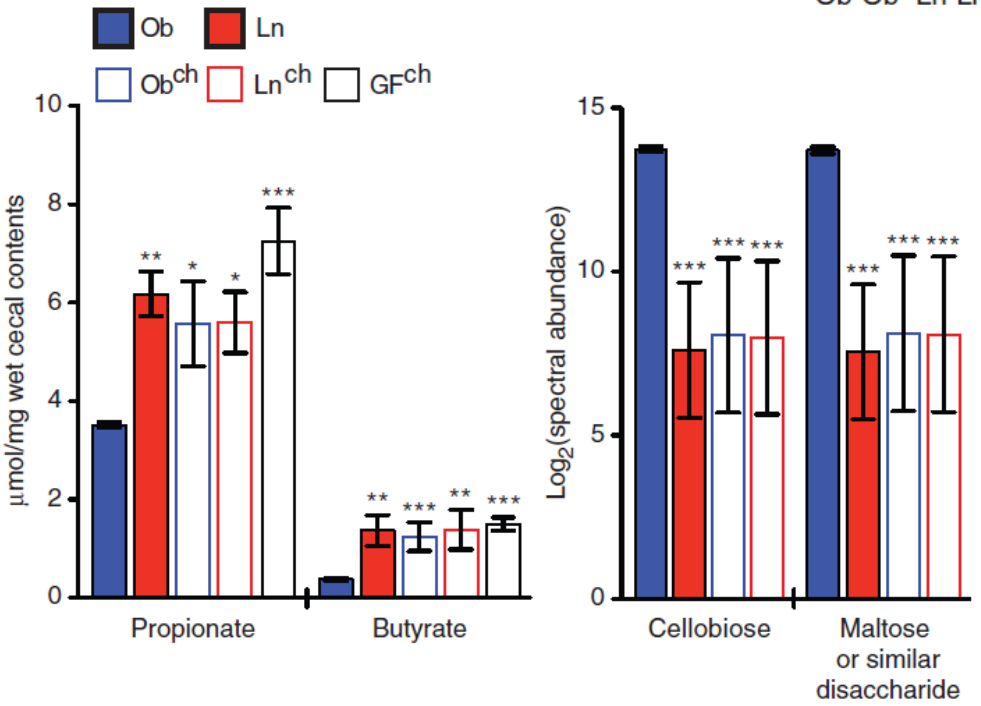


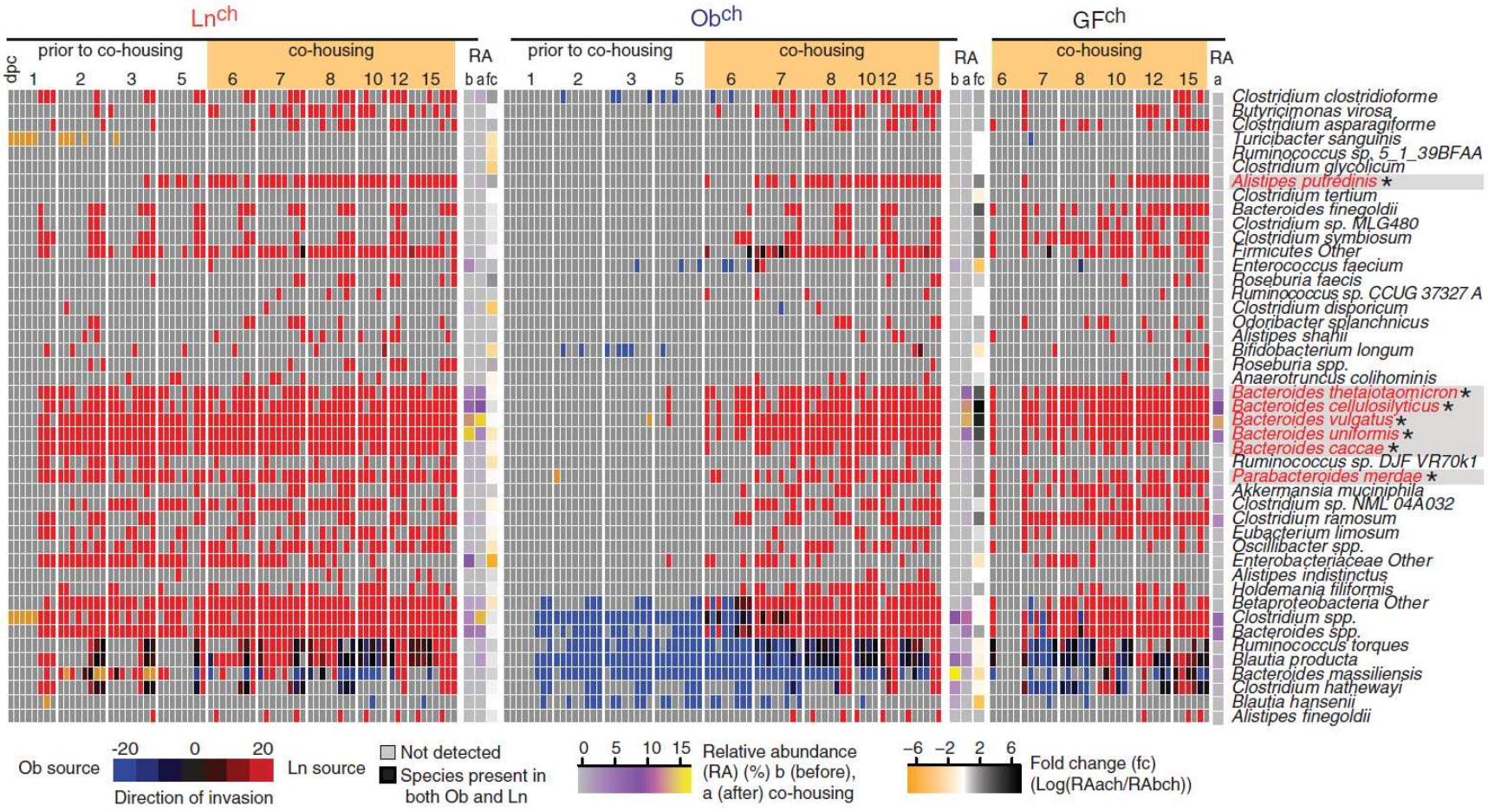
dpc, days post colonization

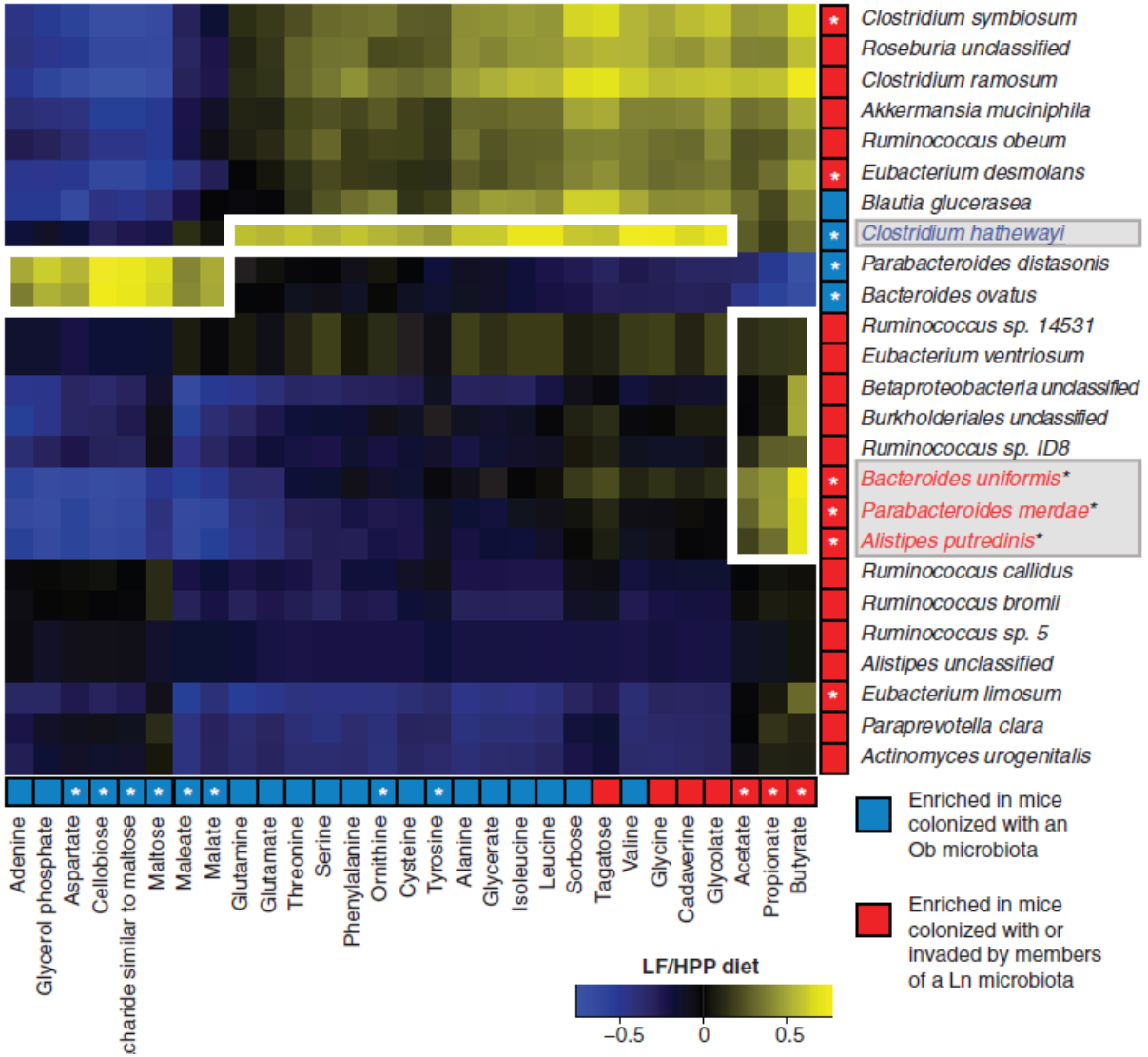


GF, germ free mice



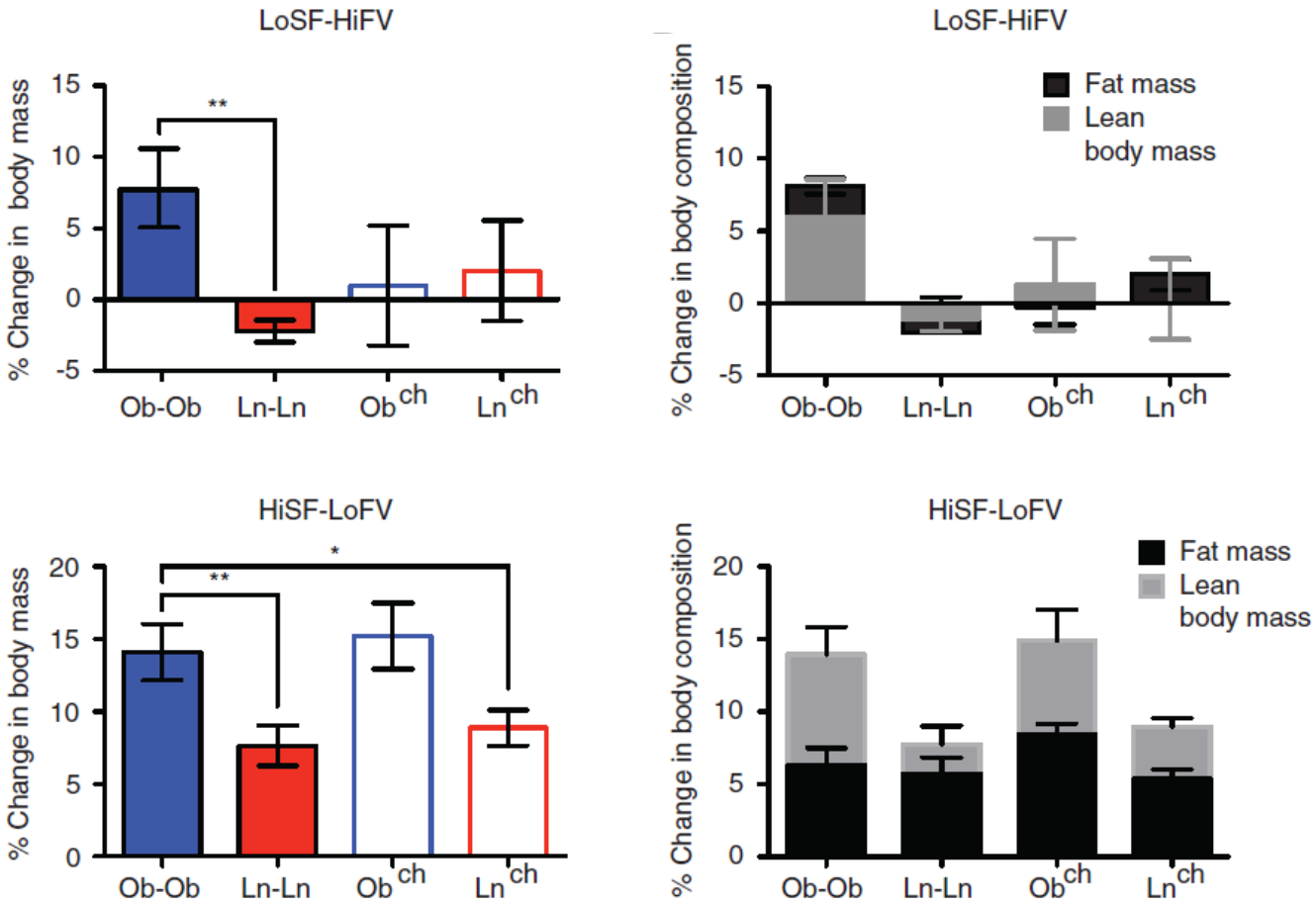



Ln^ch
Ob^ch
GF^ch
prior to co-housing
co-housing
dpc
1 2 3 5 6 7 8 10 12 15
RA
b a fc
Clostridium clostridioforme
Butyricimonas virosa
Clostridium asparagiforme
Turicibacter sanguinis
Ruminococcus sp. 5_1_39BFAA
Clostridium glycolicum
Alistipes putredinis *
Clostridium tertium
Bacteroides finegoldii
Clostridium sp. MLG480
Clostridium symbiosum
Firmicutes Other
Enterococcus faecium
Roseburia faecis
Ruminococcus sp. CCUG 37327 A
Clostridium disporicum
Odoribacter splanchnicus
Alistipes shahii
Bifidobacterium longum
Roseburia spp.
Anaerotruncus colihominis
Bacteroides thetaiotaomicron *
Bacteroides cellulosilyticus *
Bacteroides vulgatus *
Bacteroides uniformis *
Bacteroides caccae *
Ruminococcus sp. DJF VR70k1
Parabacteroides merdae *
Akkermansia muciniphila
Clostridium sp. NML 04A032
Clostridium ramosum
Eubacterium limosum
Oscillibacter spp.
Enterobacteriaceae Other
Alistipes indistinctus
Holdemania filiformis
Betaproteobacteria Other
Clostridium spp.
Bacteroides spp.
Ruminococcus torques
Blautia producta
Bacteroides massiliensis
Clostridium hathewayi
Blautia hansenii
Alistipes finegoldii
Ob source
-20 0 20
Direction of invasion
Ln source
Not detected
Species present in both Ob and Ln
0 5 10 15
Relative abundance (RA) (%) b (before), a (after) co-housing
−6 −2 2 6
Fold change (fc) (Log(RAach/RAbch))

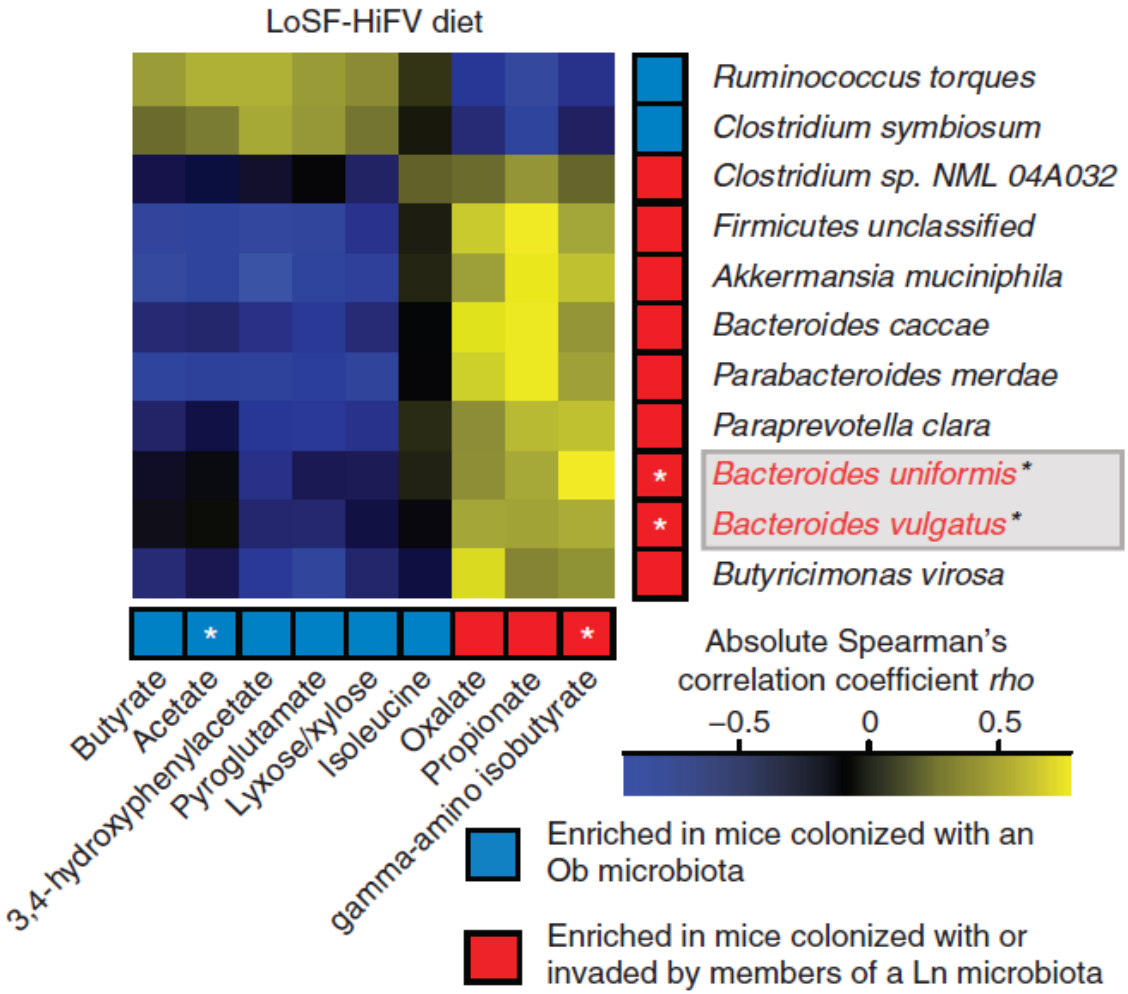


LF-HPP, low in fat (4% by weight) and high in plant polysaccharides

Diet-Specific Effects



LoSF-HiFV, low–saturated fat, high fruits and vegetables
HiSF-LoFV, saturated fats and the lower of fruits and vegetables


LoSF-HiFV diet
Ruminococcus torques
Clostridium symbiosum
Clostridium sp. NML 04A032
Firmicutes unclassified
Akkermansia muciniphila
Bacteroides caccae
Parabacteroides merdae
Paraprevotella clara
Bacteroides uniformis*
Bacteroides vulgatus*
Butyricimonas virosa
Butyrate
Acetate
3,4-hydroxyphenylacetate
Pyroglutamate
Lyxose/xylose
Isoleucine
Oxalate
Propionate
gamma-amino isobutyrate
Absolute Spearman's correlation coefficient rho
–0.5
0
0.5
Enriched in mice colonized with an Ob microbiota
Enriched in mice colonized with or invaded by members of a Ln microbiota

Culture collections generated from human microbiota samples can transmit donor phenotypes of interest (body composition and metabotypes).

If these derived culture collections can transmit a phenotype, the stage is set for studies designed to determine which culturable components of a given person's gut community are responsible.

Sequenced culture collections generated from human gut microbiota donors also provide an opportunity to model and further address basic issues such as the determinants of invasiveness including the mechanisms by which invasion is impacted by diet composition, as well as the mechanisms by which invading components affect microbial and host metabolism.

This issue is important for identifying next-generation probiotics, prebiotics, or a combination of the two (synbiotics).

Moreover, the ability to generate a culture collection, from an individual—whose composition is resolved to the gene level, whose properties can be validated in preclinical models, and whose "manufacture" is reproducible—may provide a safer and more sustainable alternative to fecal transplants for microbiome-directed therapeutics

Fusobacterium nucleatum Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment

Cell Host & Microbe
Short Article

Fusobacterium nucleatum
Potentiates Intestinal Tumorigenesis and Modulates the Tumor-Immune Microenvironment

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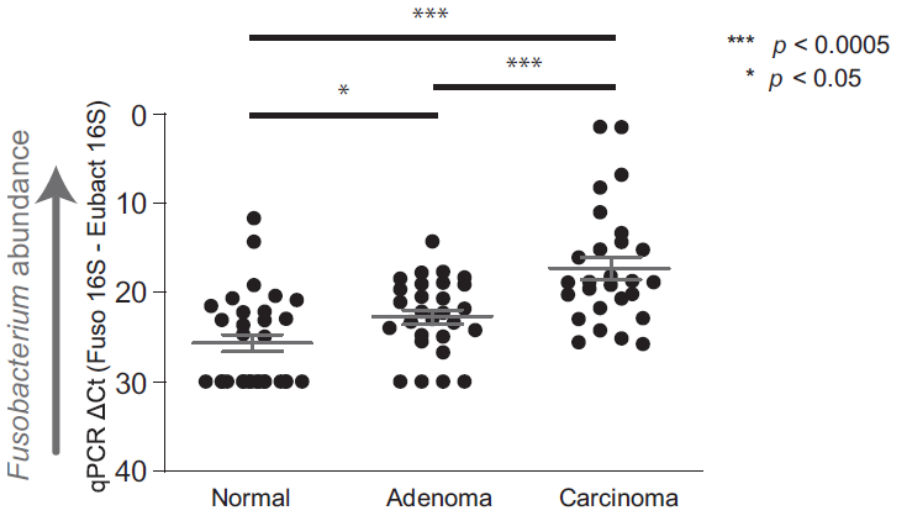
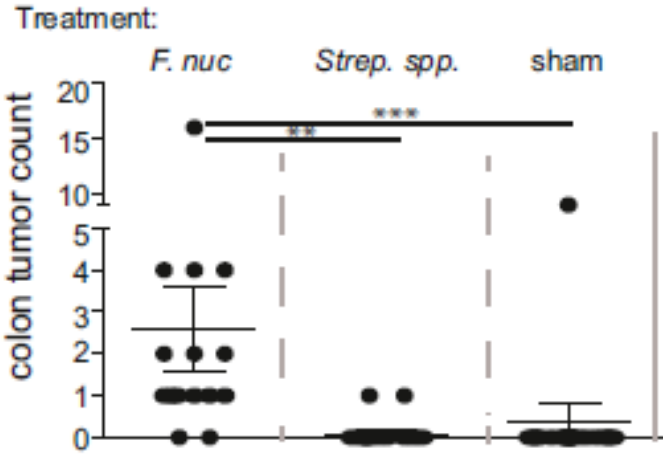
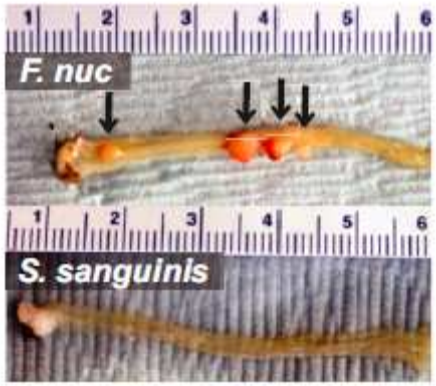
http://dx.doi.org/10.1016/j.chom.2013.07.007

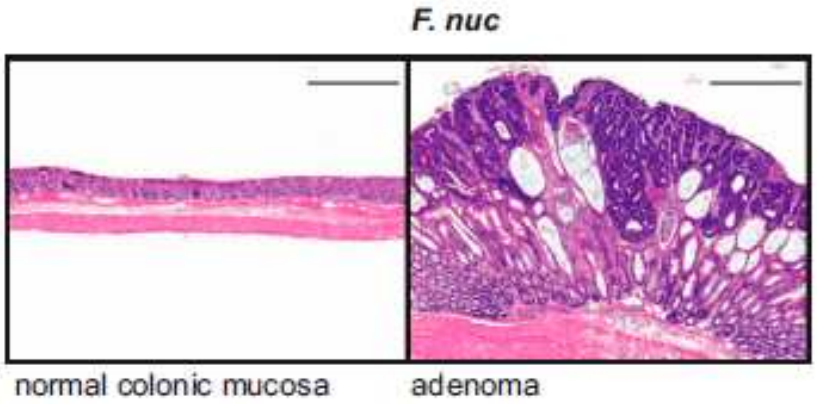
Metagenomic analyses indicate that symbiotic *Fusobacterium* spp. are **associated with human colorectal carcinoma**,
but whether this is an indirect or causal link remains unclear.
We find that *Fusobacterium* spp. are enriched in human colonic adenomas relative to surrounding tissues and in stool samples from colorectal adenoma and carcinoma patients compared to healthy subjects.





In the ApcMin/+ mouse model of intestinal tumorigenesis, *Fusobacterium nucleatum* increases tumor multiplicity and selectively recruits tumor-infiltrating myeloid cells, which can promote tumor progression.





Although barrier defects expose the intestinal mucosa to the entire luminal microbial milieu, *Fusobacterium* spp. Become the most highly enriched bacterium in colorectal tumors relative to adjacent tissue.

This enrichment may be attributable to the **strong adhesive and invasive** abilities of fusobacteria for epithelial cells.

Alternatively, **metabolic specializations** may endow fusobacteria with a competitive advantage in the evolving tumor milieu. *Fusobacterium nucleatum* is an asaccharolytic bacterium; therefore, unlike the Enterobacteriaceae, it will not compete for glucose, a preferred substrate for tumor metabolism.

Instead, fusobacteria can utilize **amino acids** and **peptides** as nutrient sources in the tumor microenvironment.

In addition, *F. nucleatum* strains, unlike many strict anaerobes of the intestinal lumen, possess a **rudimentary electron transport chain**, endowing them with a limited ability to respire oxygen. Thus, *F. nucleatum* may be able to persist and slowly replicate in the hypoxic tumor microenvironment.

Adhesive molecules that contribute to invasiveness in *F. nucleatum* can promote bacterial aggregation and biofilm formation that also enhance oxygen tolerance. Products of fusobacterial metabolism may make the tumor microenvironment more tumor permissive over time by directly promoting tumor cell proliferation, blood vessel growth, or immune cell infiltration.