

# Mechanism of Action of Probiotics

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# Conflict of Interest Statement

No conflict of interest for material  
in this presentation

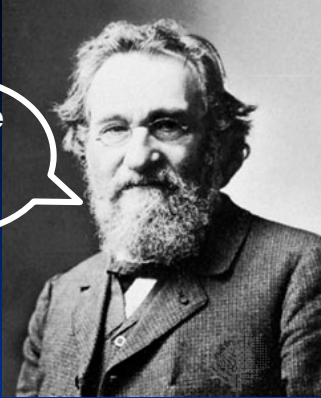
- Given lectures sponsored by companies commercialising probiotics eg Nestle Nutrition Institute, Danone and Yakult
- Developed probiotic based products eg supplements and foods for food and biotech companies
- Currently Chief Scientist for ProBiOz P/L

# Overview

- Historical perspective
- Gut microbiota
- Concept of probiotics?
- Probiotics for infections
- Probiotics for non-infectious diseases
- Limitations with identifying mechanisms

# Historical Perspective

Are we there yet?



Lactobacillus used for a HUGE range of conditions



**Conflicting outcomes**; Poorly defined products/preparations; controversy



Antibiotics developed; probiotics not needed



Probiotics for **digestive tract** uses only  
Eg Lactobacillus and Bifidobacterium



Improved methods for studying gut microbes; Probiotic applications increase dramatically with defined studies and models for testing.



Reduce intestinal putrefaction;  
**Eat yoghurt**  
Increase longevity (1907)  
Nobel prize – macrophages

**Gut microbes and health; long list of potential benefits; gut focus**

# Microbiota and the emerging pandemic of NCDs (Non-Communicable Diseases)

- Early life exposures**
- Mode of delivery
  - Infant diet
  - Antibiotic usage
  - Environmental factors

- Life style choices**
- Diet
  - Medications
  - Stresses



- Immune**  
atopy, asthma,  
multiple sclerosis,  
respiratory

- Intestinal**  
Inflammatory bowel disease  
Diarrhoea; IBS  
Necrotising enterocolitis  
Colon cancer

- Immune tolerance**  
**Intestinal homeostasis**  
**Healthy metabolism**

- Metabolic; Liver**  
Diabetes, obesity

- Mental; neurological**

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Not just targeting the microbiome

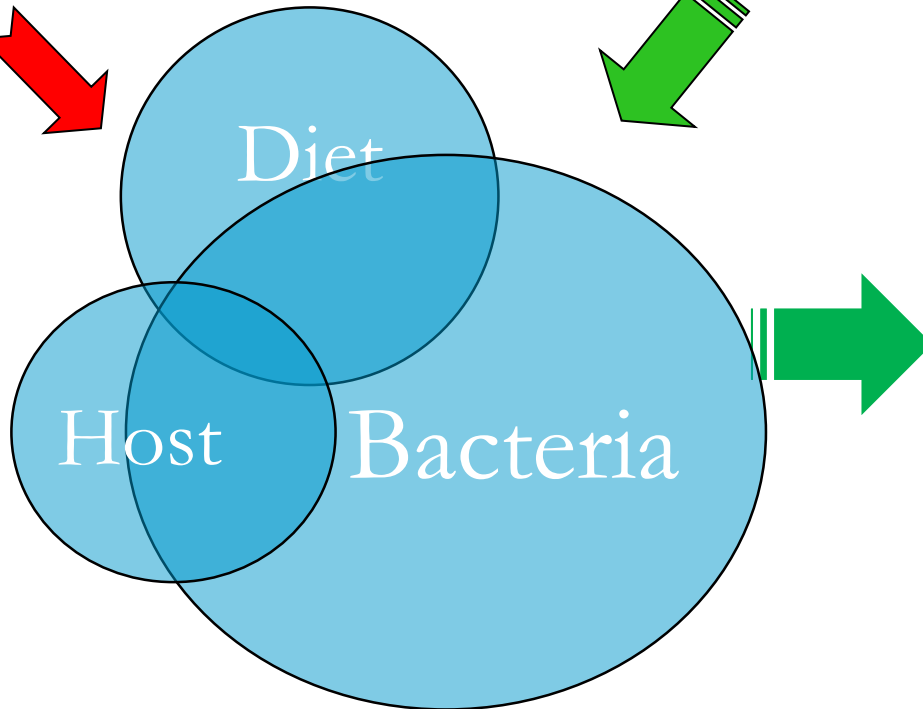
# Possible actions of some probiotic strains.

## Stress

- emotional
- dietary
- 
- medications
- physical
- ageing



## Probiotics



### Improved digestive health

Less

- infection
- disease
- constipation
- diarrhea

### Reduced metabolic disorders

Less

- obesity
- diabetes

### Improved immune modulation

Reduced

- infection
- inflammation

### Neurological improvement

- improved function
- new nerve cell growth
- less anxiety

# Probiotics

Most common genera:

*Lactobacillus*

*Bifidobacterium*

Most common species:

*Lactobacillus acidophilus*

Genus species STRAIN:

e.g.

*Lactobacillus acidophilus* GG

Pure  
culture

Mixed  
culture

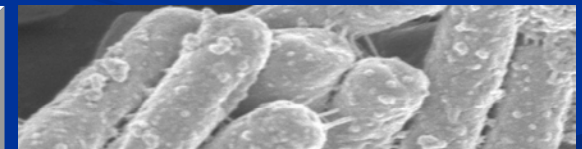
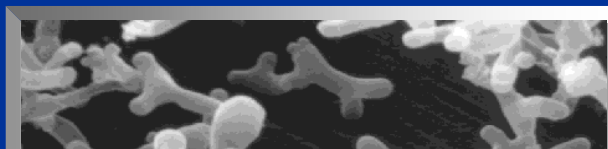
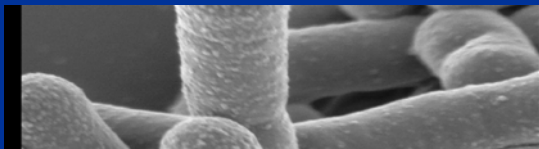
*Bacillus  
coagulans*

*E. coli*

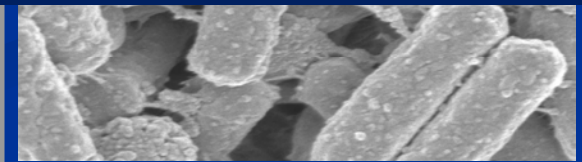
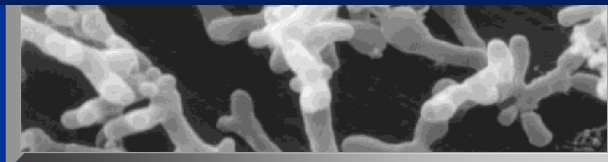
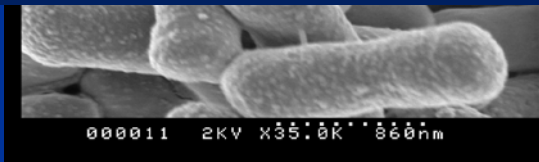
*Saccharomyces  
boulardii*

*Faecalibacterium  
prausnitzii*

*Clostridium  
sporogenes*



Different preparations – different mechanisms of action





# What is a probiotic?

- FAO/WHO definition

(live, survive low pH and bile, colonize by adhesion; beneficial)

- Predominately lactobacillus and bifidobacteria
- What about *Saccharomyces boulardii*?
- What about spore forming *Bacillus* spp or *Clostridium* spp?
- What about heat killed bacterial preparations?
- Single strains or mixed strains
- Next generation probiotics eg *Faecalibacterium prausnitzii*
- Faecal homogenates?

# Mode of action

## Prophylactic or therapeutic?

### Direct effect on:

- a pathogen  
(bacterial/viral/protozoal)
- the host immune system
- the host physiology
- enzymatic capacity
- drug metabolism

### Indirect effects on:

- Gut microbiome
- Host immune modulation
- Host physiology
- Respiratory conditions
- Metabolic diseases
- Neurological conditions
- Cancers

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# Benefits: impact on infection

(bacterial; viral; protozoal)

## Inhibition of pathogen growth

- pH, organic acids, H<sub>2</sub>O<sub>2</sub>, peptides (AMPs)

## Inhibition of pathogen adhesion

- block receptor (steric hindrance; specific receptor)

## Competition for nutrients

- outcompete pathogen for essential nutrients

## Immune triggering

- Trigger IgG and IgA secretion



Inhibition of  
*E.coli* ETEC

Salmonella	Probiotic A	Probiotic B
Growth inhibition	6%	71%
Adhesion inhibition	6 times	100 times
Weight change of mice	-7%	+0.8%

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# Benefits: non-infectious (non-communicable diseases)

## Modulation of inflammation

- immune receptor cascade signalling

## Neurological signals to and from the gut

## Enhanced barrier function of the gut epithelium

- Enhanced mucin production
- Enhanced integrity

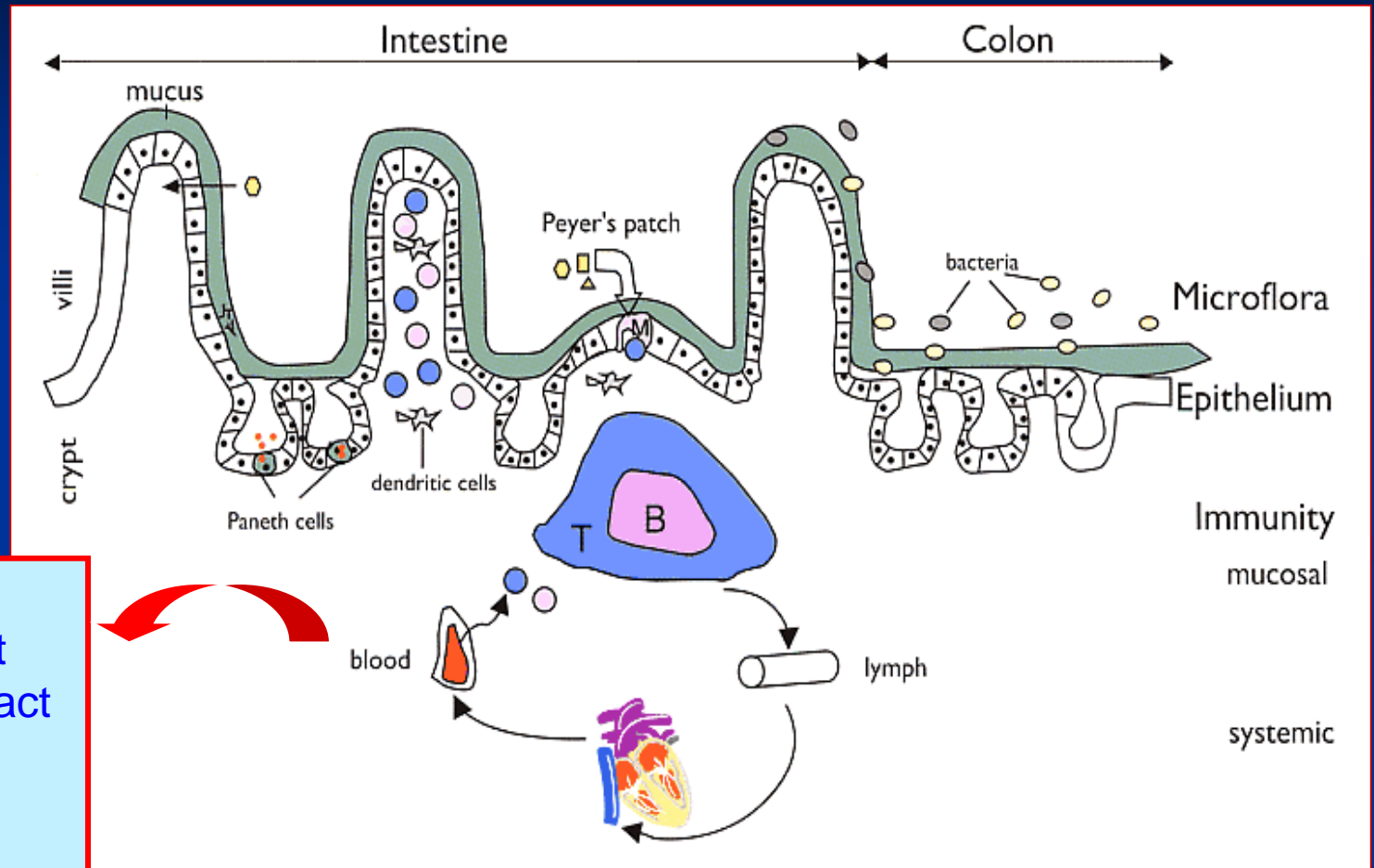
## Reduced risk of cancer

- apoptosis related enzymes;
- prevent enzymatic co-carcinogen conversion
- Immune responses

## Outcompete antimicrobial resistant (AMR) bacteria

- Microbiome targets

# Gut-associated lymphoid tissues (GALT) Adaptive Immunity



## Mucosal Site

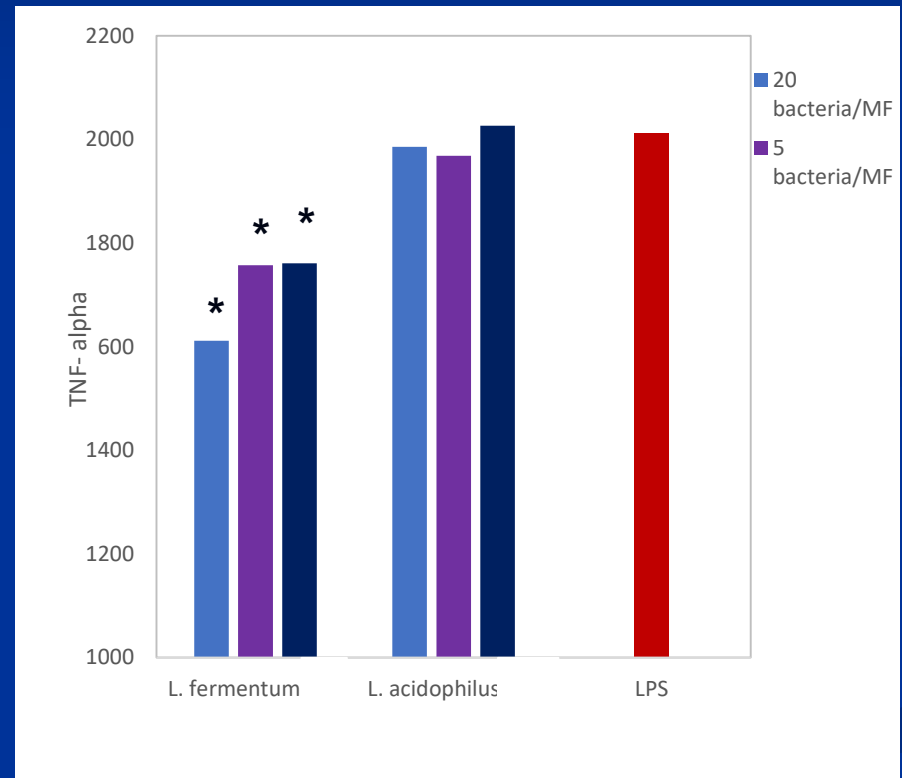
- Digestive tract
- Respiratory tract
- Nasal cavity
- Vaginal tract
- Urinary tract
- Mammary glands
- Skin

## Activation of T cells and B cells

Less IgE (less allergy); More IgA and IgG (less infection);  
Altered cytokines (less inflammation)

# Modulation of lipopolysaccharide (LPS) inflammation

- Gut inflammation commonly caused by LPS
- Attenuated TNF- $\alpha$  production in both prophylactic and therapeutic models





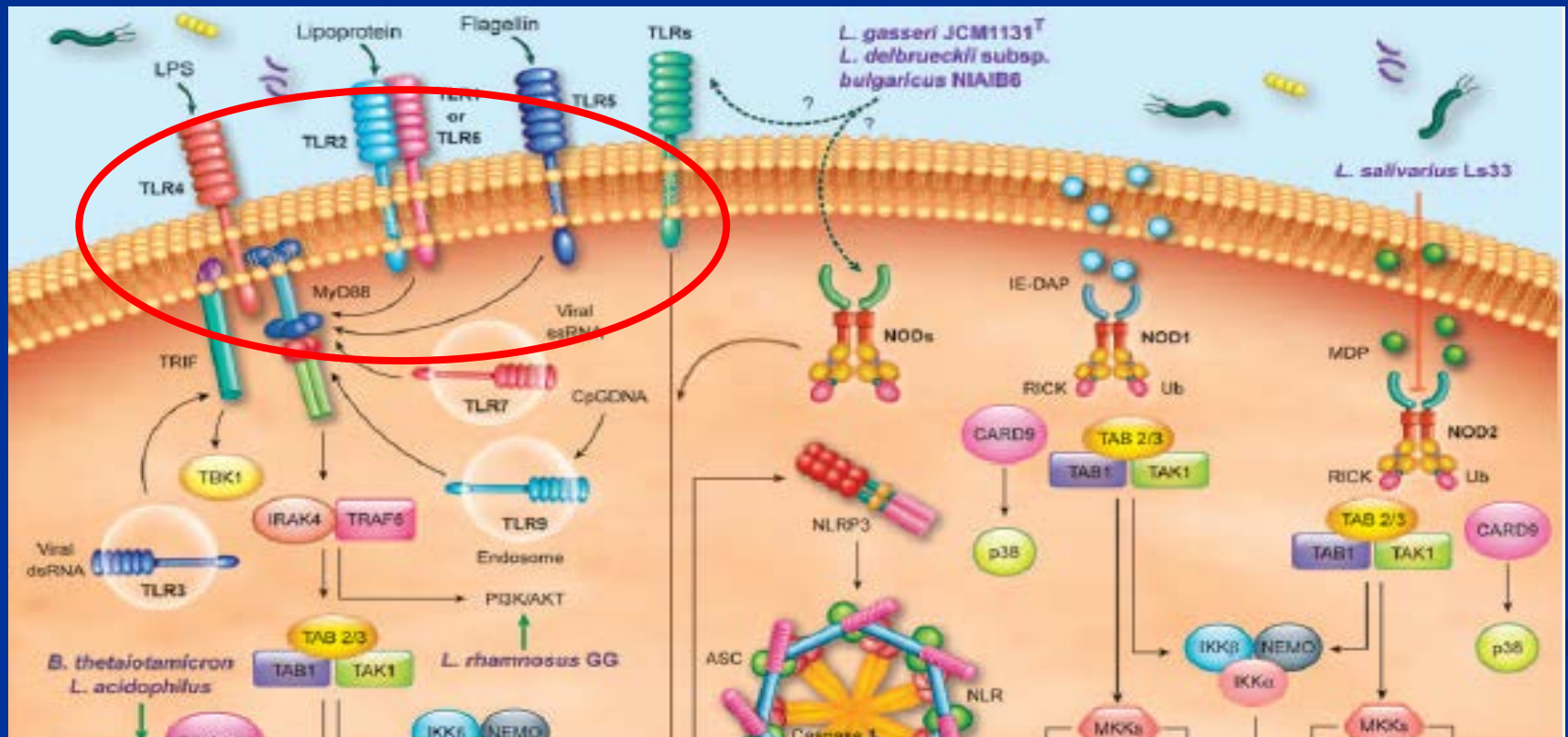
# Innate Immunity

(responds to pathogen associated molecular patterns ie PAMPs)

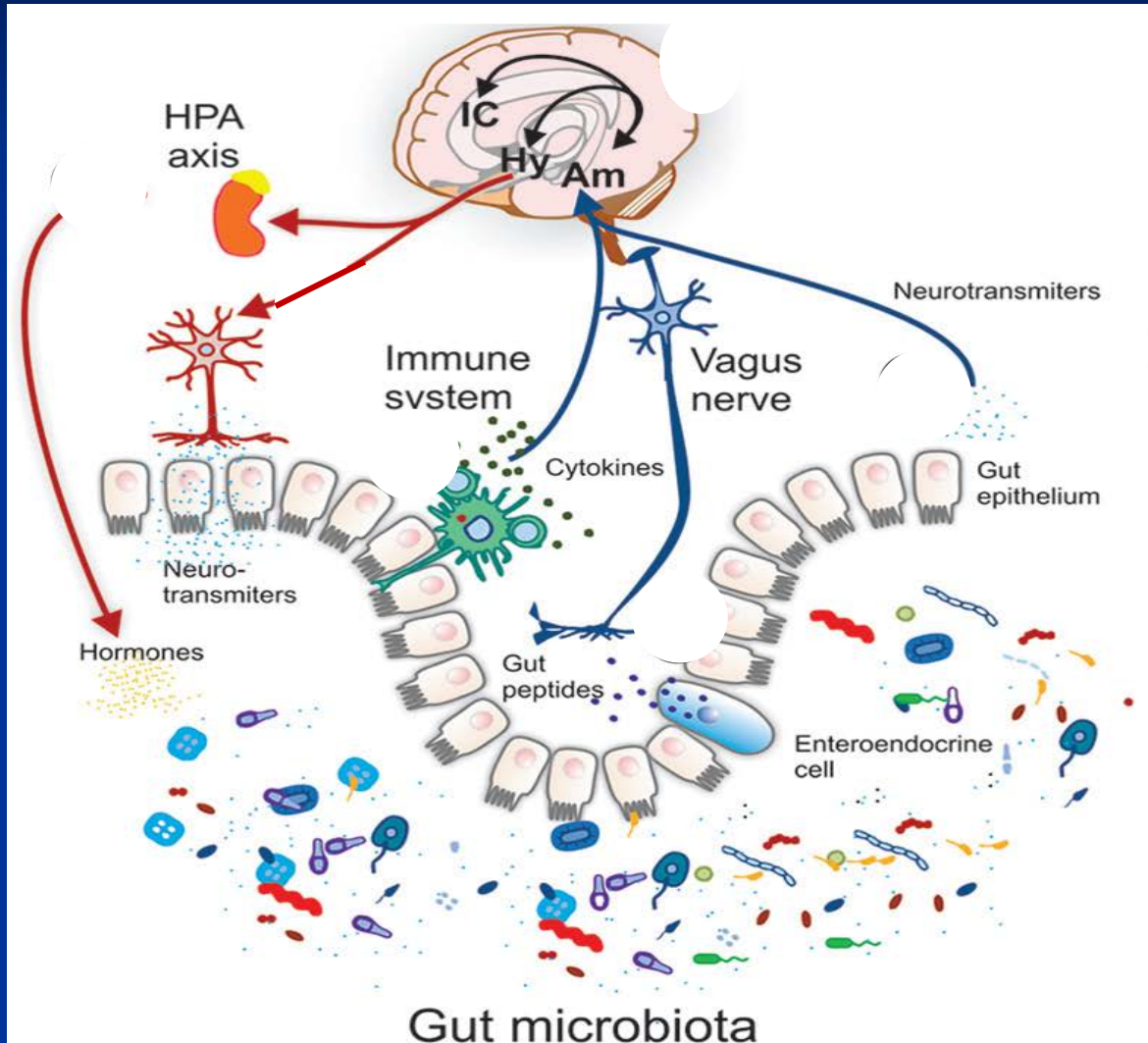
Pattern recognition receptors PPRs  
eg Toll-like receptors (TLRs and NOD 2)

Bermudez et al

Ann Nutr Metab 2012;61:160-174  
DOI: [10.1159/000342079](https://doi.org/10.1159/000342079)



# Gut-brain axis (GBA).



# Overview

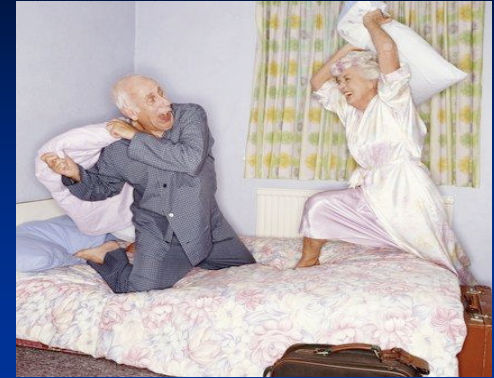
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# Methods for studying mechanisms

- “Omics” – who is there and what are they doing (metagenomics; transcriptomics; metabolomics; proteomics)
- Host physiology (biological sampling)
- *In vitro* and *ex-vivo* models
- Tissue culture (2D and 3D) and organoids
- Animal models (germ free, knock-out mice and SPF)
- *In vivo* data correlations with *in vitro* studies

# Factors Impacting on Probiotic Actions

- Medications
- Diet
- Stressors
- Age
- Dental health
- Infection
- Hygiene
- Sanitization
- Urban/rural
- Genetics
- Environment
- Exercise



# Cautions & Limitations

- Need to define strains
- Models for studying mechanisms (*in vitro* assays, animals)
- Models provide evidence of mechanism (pathways affected)
- Extrapolation from models has limits
- Need measurable effects
- Understand limitations of clinical studies  
(number and diversity of subjects)
- More than just fixing the microbiome  
( host physiology and immune aspects)

# Understanding modes of action: – allows understanding outcomes

## Multicenter Trial of a **Combination Probiotic** for Children with Gastroenteritis

Stephen B. Freedman, M.D.C.C.M., Sarah Williamson-Urquhart, B.Sc.Kin., Ken J. Farion, M.D., Serge Gouin, M.D.C.C.M., Andrew R. Willan, Ph.D., Naveen Poonai, M.D., Katrina Hurley, M.D., Philip M. Sherman, M.D., Yaron Finkelstein, M.D., Bonita E. Lee, M.D., Xiao-Li Pang, Ph.D., Linda Chui, Ph.D., David Schnadower, M.D., M.P.H., Jianling Xie, M.D., M.P.H., Marc Gorelick, M.D., and Suzanne Schuh, M.D.et al., for the PERC PROGUT Trial Group\*

November 22, 2018  
N Engl J Med 2018; 379:2015-2026  
DOI: 10.1056/NEJMoa1802597

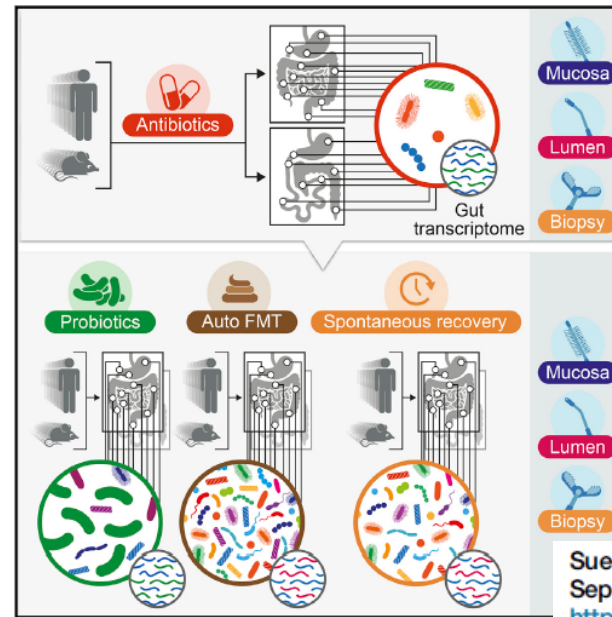
## **Lactobacillus rhamnosus GG** versus Placebo for Acute Gastroenteritis in Children

David Schnadower, M.D., M.P.H., Phillip I. Tarr, M.D., T. Charles Casper, Ph.D., Marc H. Gorelick, M.D., M.S.C.E., J. Michael Dean, M.D., Karen J. O'Connell, M.D., Prashant Mahajan, M.D., M.P.H., Adam C. Levine, M.D., M.P.H., Seema R. Bhatt, M.D., Cindy G. Roskind, M.D., Elizabeth C. Powell, M.D., Alexander J. Rogers, M.D., et al.

November 22, 2018  
N Engl J Med 2018; 379:2002-2014  
DOI: 10.1056/NEJMoa1802598

## Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT

### Graphical Abstract



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### In Brief

Probiotics perturb rather than aid in microbiota recovery back to baseline after antibiotic treatment in humans.

Suez et al., 2018, Cell 174, 1406–1423  
September 6, 2018 © 2018 Elsevier Inc.  
<https://doi.org/10.1016/j.cell.2018.08.047>

Abstract

# Conclusions

- Strain dependant effects
- Benefits can be direct or indirect
- Can impact on infection agents
- Can influence non-infectious diseases (immune triggereing)
- Can have prophylactic and/or therapeutic effects



**Thank you**

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