Modulation of gut microbiome in prevention and treatment of chronic diseases

- RNDr. Jana Štofilová, PhD.

iEM INSTITUTE OF EXPERIMENTAL MEDICINE

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MediTec

1.-15.9.2019, MediTec Training for Students, UPJŠ, Košice, Slovakia

Overview

Institute of Experimental Medicine

- Research team
- Infrastructure
- Long-term research program

The role of gut microbiota in health and disease

- Factors affecting the composition of gut microbiota

Modulation of gut microbiota in diseases

Probiotics and prebiotics

Fecal microbiota transplantation

Project APVV TRANSMICROBIOM

INSTITUTE OF EXPERIMENTAL MEDICINE

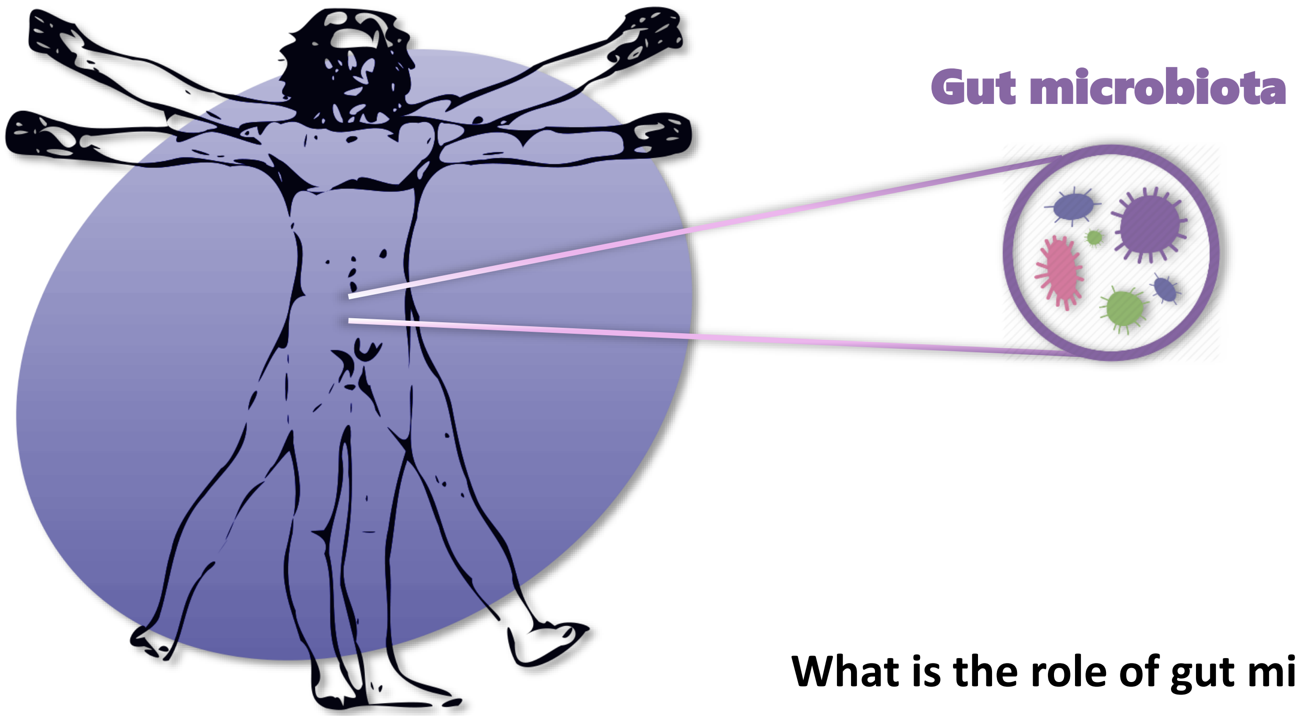
RESEARCH TEAM

GROUP LEADER:

Alojz BOMBA, DVM, DSc

- 6 senior scientists
- 5 junior scientist
- 3 PhD students
- 3 technicians

Institute of Experimantal Medicine



Key questions:

What is the role of gut microbiome in pathogenesis of chronic diseases?

What are possibilities for prevention of chronic diseases using targeted modulation of gut microbiome?

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OUR EXPERITISE

PROBIOTICS & PREBIOTICS

HUMAN GUT MICROBIOME & CHRONIC DISEASES

METHODS

MODELS

In vivo rat models (colitis, cancer, dysbiosis)

In vitro model of human GIT (SHIME®)

Cell culture models

ANALYSIS

MICROBIOME (PCR-DGGE, qPCR, NGS)

SERUM PARAMETERS

GAS CHROMATOGRAPHY

FLOW CYTOMETRY

Infrastructure

Infrastructure

INSTITUTE OF
EXPERIMENTAL
MEDICINE

Molecular biology Lab

Microbiology Lab

Cell culture Lab

Biochemistry Lab

Mass Spectrometry Lab

Immunology Lab

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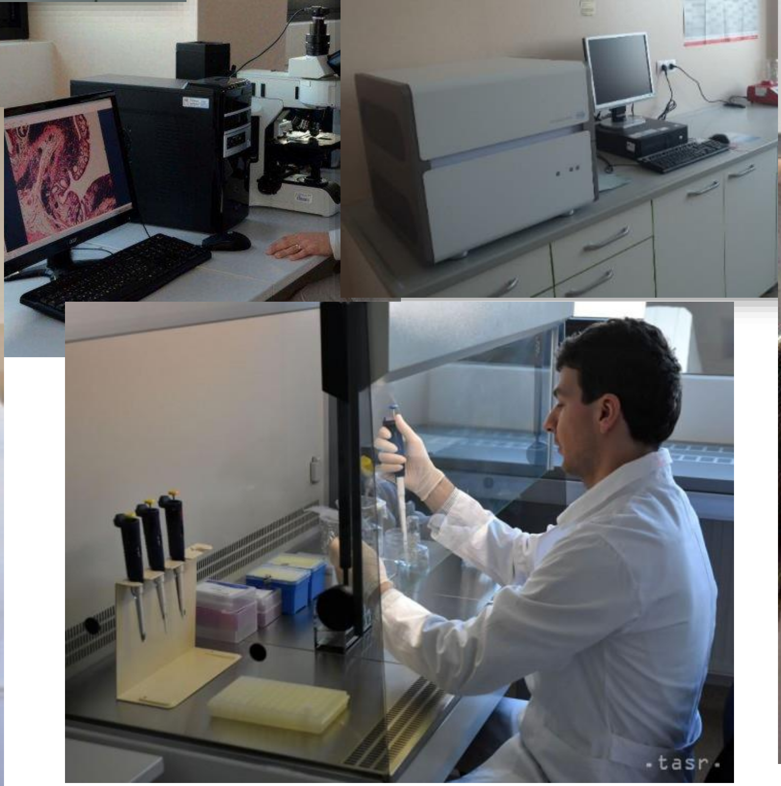
INFRASTRUCTURE





TWINSHIME®
Simulator of Human Intestinal Microbial Ecosystem

CONFERENCE ORGANISATION

The 40th International Congress of the Society for Microbial Ecology and Diseases
(SOMED 2018, Hungary)

International Scientific Conference on Probiotics and Prebiotics
(IPC 2008-2013 Slovakia, IPC 2014-2018 Hungary, IPC 2019 Czech Republic)

International Scientific Conference on Functional Foods
(Food and Function 2009, 2011 Slovakia)

International Scientific Conference of Society for Microbial Ecology and Disease
(SOMED 2013, Slovakia)

International Scientific Conference GutMicroEcology
(GME 2010, Slovakia)

European Researchers' Night

Human Microbiome project

- The overall **mission of the HMP was** to generate resources to facilitate **characterization of the human microbiota to further our understanding of how the microbiome impacts human health and disease**.

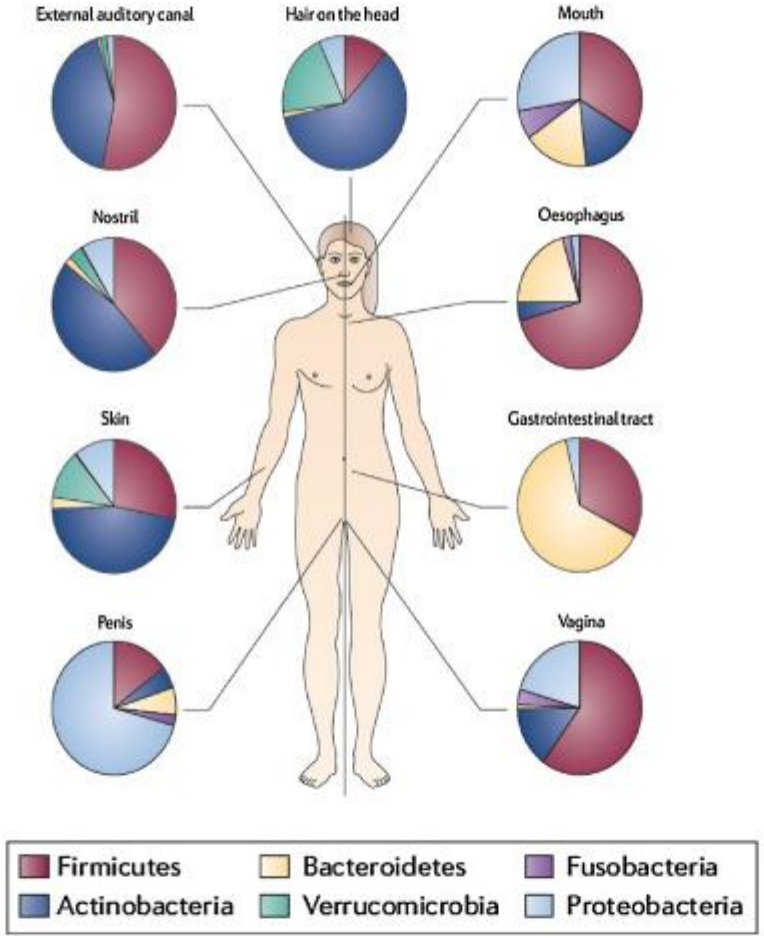
- The initial phase of the project, HMP1, established in 2008, **characterized the microbial communities from 300 healthy individuals**, across several different sites on the human body: nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract, using 16S and metagenomic shotgun sequencing.

- The second phase of the HMP (iHMP, Integrative Human Microbiome Project, 2013–2016) examined **the role of the microbiome in human health and disease** through a study of three models of microbiome-related human conditions (Pregnancy & Preterm Birth, IBD and type 2 diabetes).

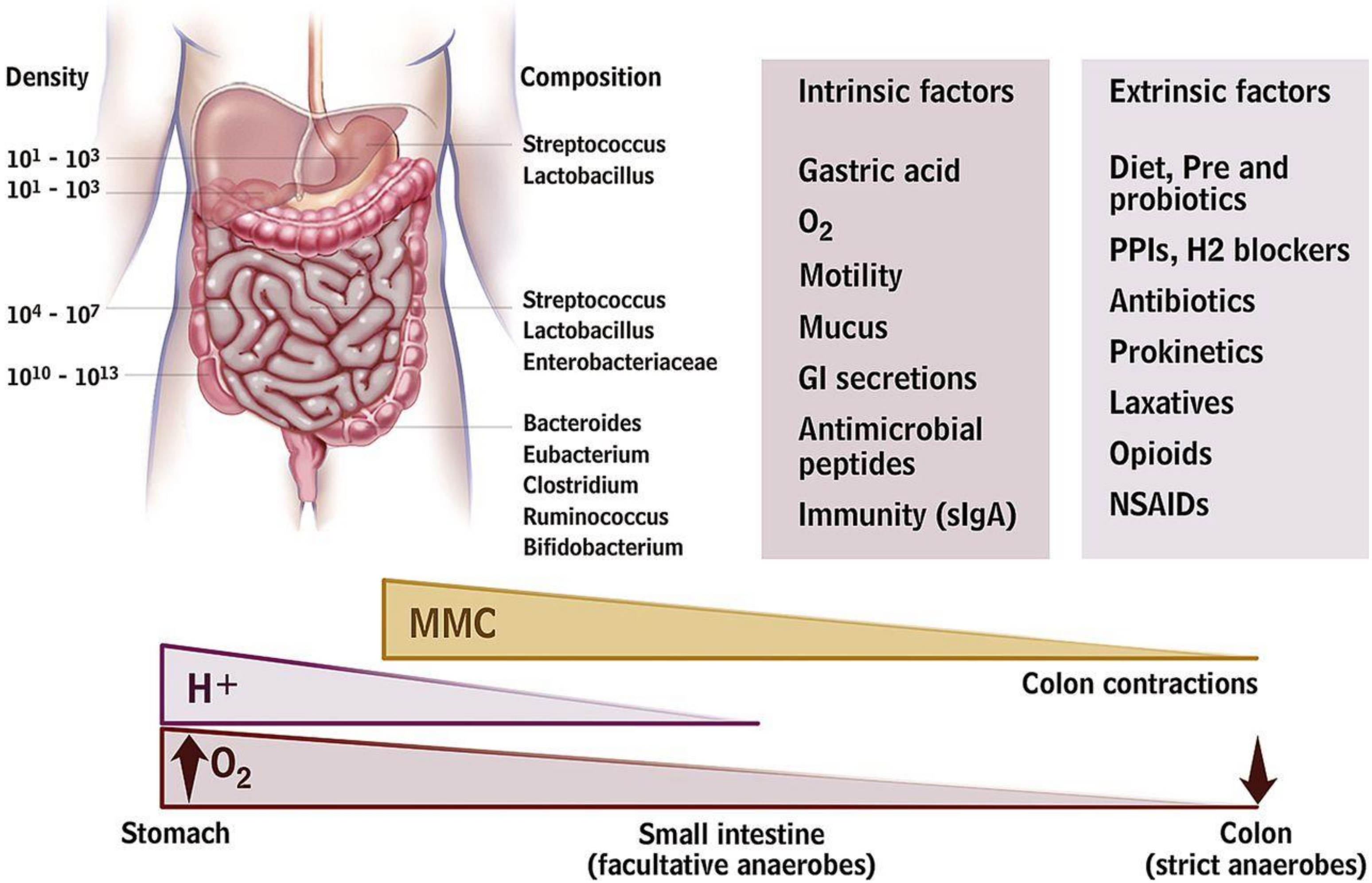
https://hmpdacc.org/

Human Microbiome

- **collection of all the microorganisms living in association with the human body**
- eukaryotes, archaea, bacteria and viruses
- **500 – 1000 different species**
- **10x more of bacteria** than human cells
- **1000 times more microbial genes** than are found in the entire human genome
- **0,9-2,7 kg bacteria in 90kg human**
- microbes are essential for maintaining health
- scientific exploration of the microbiome is in it’s infancy



Gut Microbiome



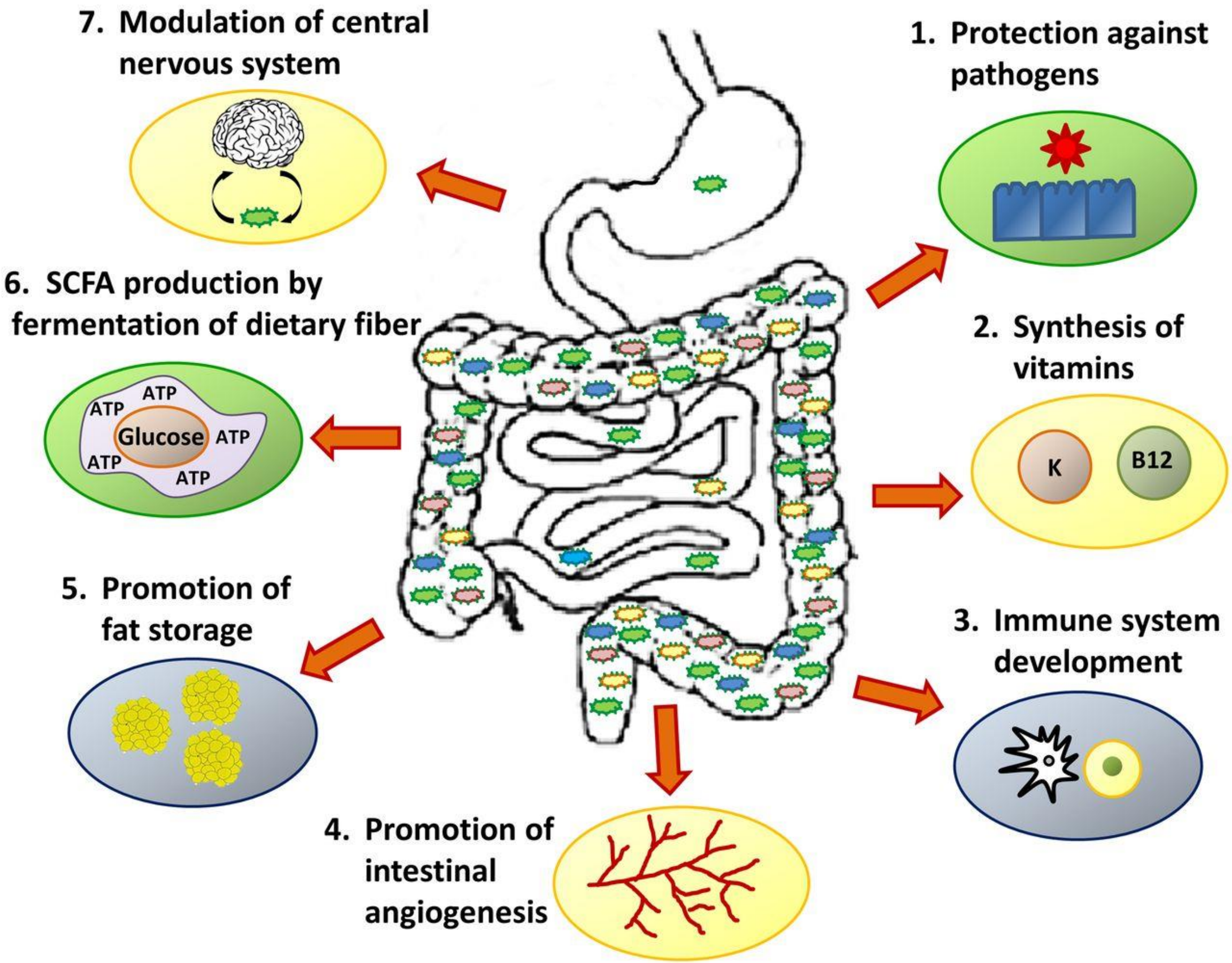
Intrinsic factors

- Gastric acid
- O_2
- Motility
- Mucus
- GI secretions
- Antimicrobial peptides
- Immunity (sIgA)

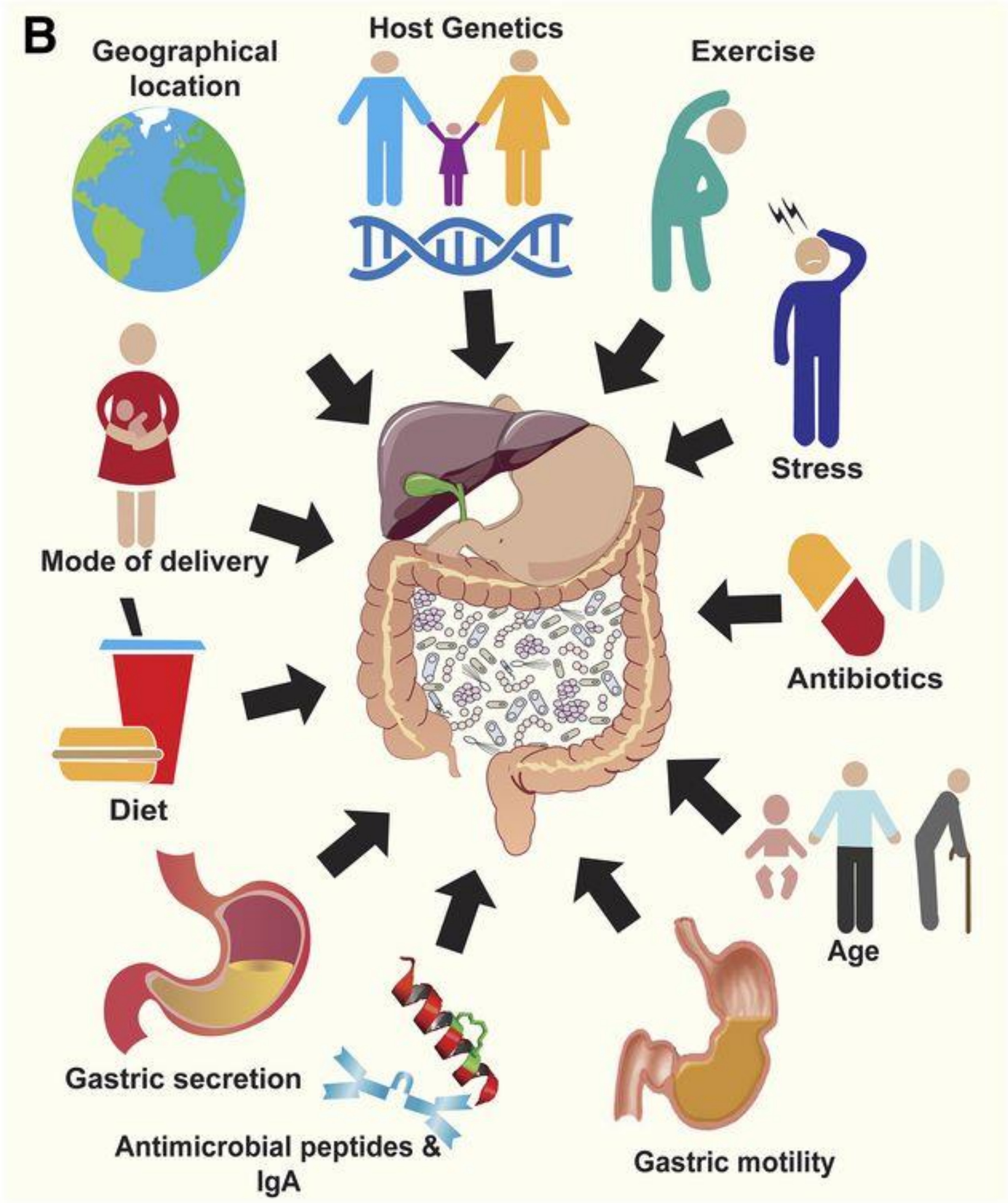
Extrinsic factors

- Diet, Pre and probiotics
- PPIs, H2 blockers
- Antibiotics
- Prokinetics
- Laxatives
- Opioids
- NSAIDs

Functions of gut microbiota

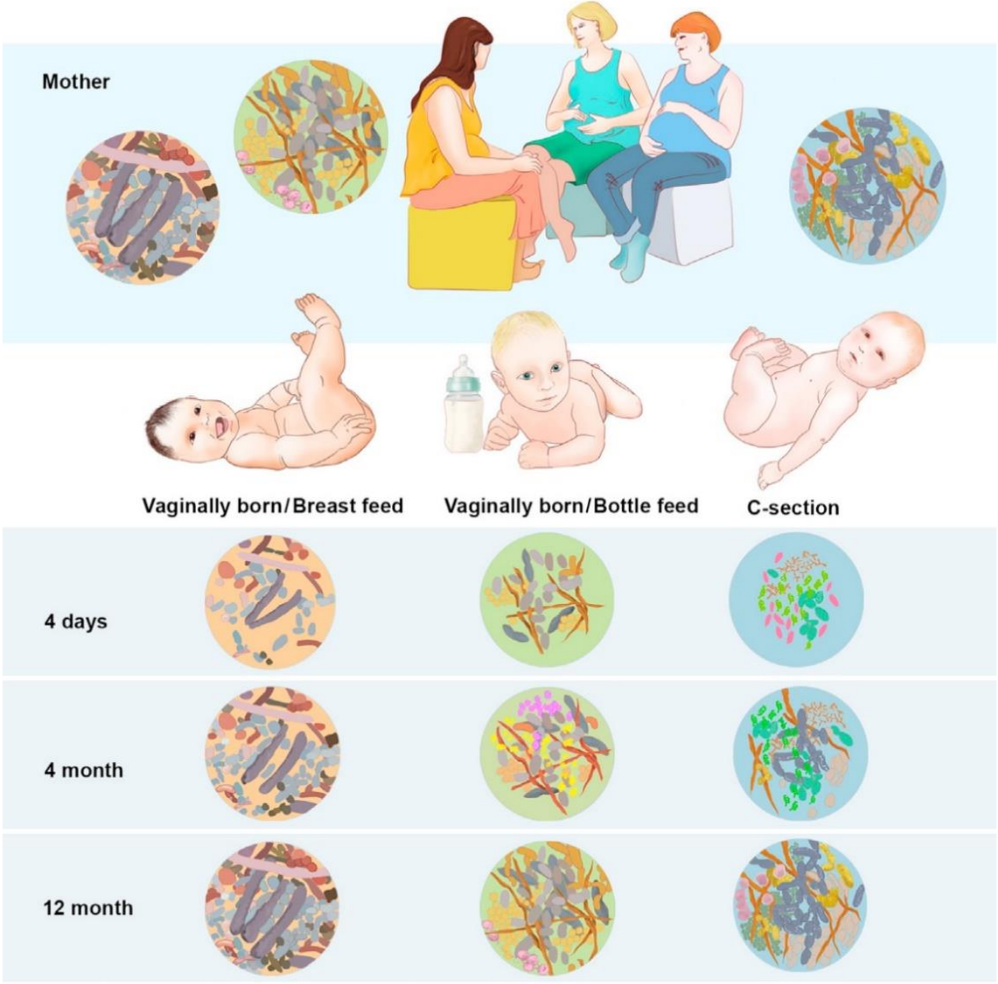


Factors affecting the composition of gut microbiome



Birth mode and infant feeding method

- Vaginally born - ↑ *Lactobacillus, Prevotella* coming from maternal vaginal tract
- C-section - ↑ *Stafylococcus, Corynebacterium, Propionobacterium, Clostridium*
- Breast feeding –dominance of *Bifidobacterium*
- Formula feeding - ↑ diversity of bacteria

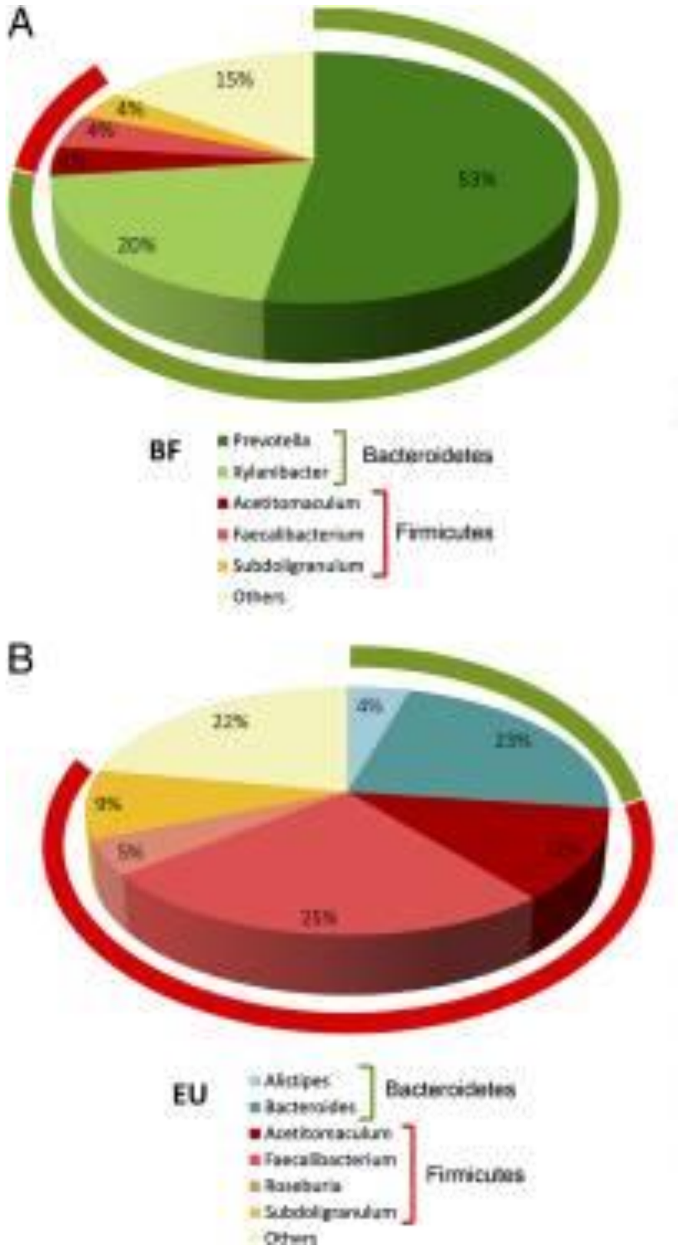


Diet

- **diet is an important driver of microbiome composition in humans**
- **gut microbiota composition differs according to diet and eating habits**
- omnivorous group has a higher diversity of bacteria compared to vegetarians

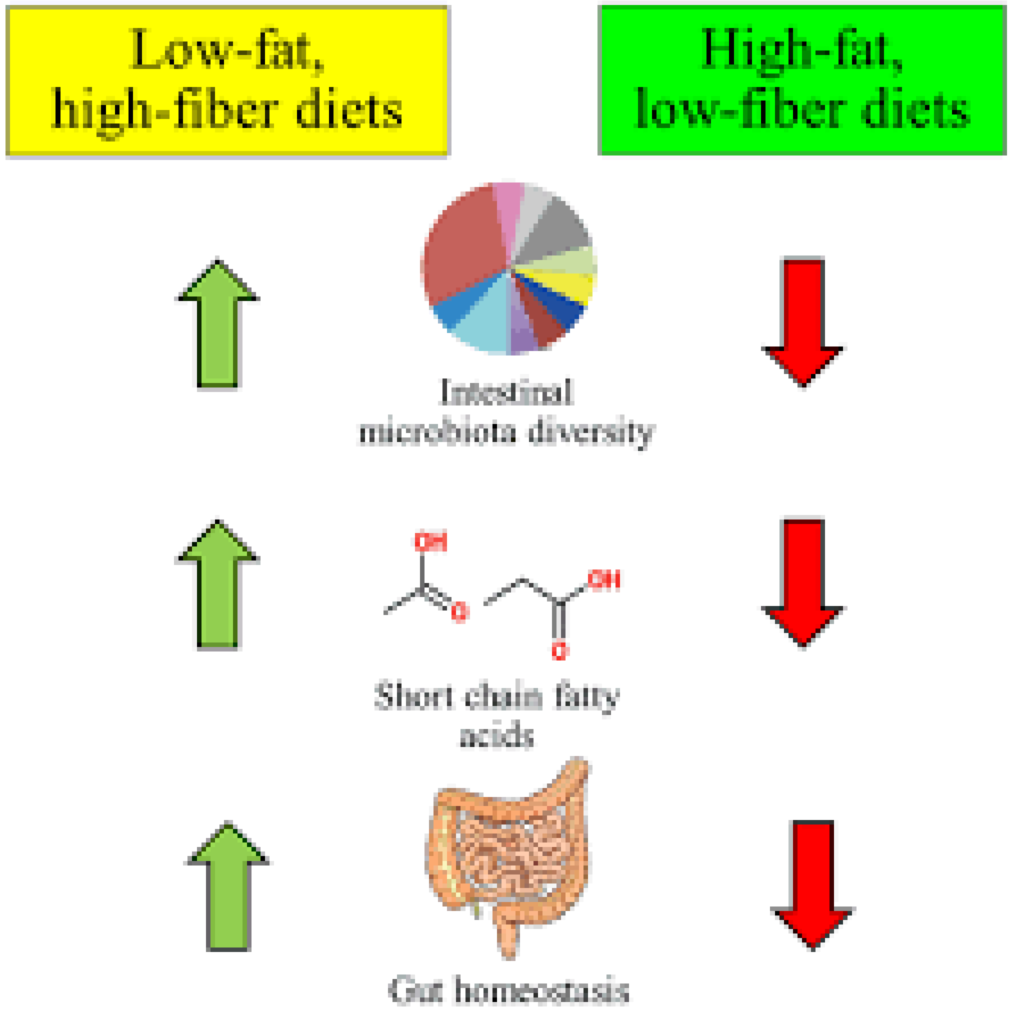
- Comparison of the intestinal microbiota of children from Africa - Burkina Faso (BF) with the microbiota of children in the EU

- Diet rich in fiber and indigestible polysaccharides leads to ↑ *Bacteroides* against *Firmicutes* in BF children



De Fillipo C. et al., Proc Natl Acad Sci U S A. 2010 Aug 17; 107(33): 14691–14696

Diet


Low-fat,
high-fiber diets
High-fat,
low-fiber diets
Intestinal
microbiota diversity
OH
O
OH
O
Short chain fatty
acids
Gut homeostasis

Enterotypes of gut microbiome

- Enterotypes are clusters of bacteria that dominate in a person’s microbiome.
- Clusters are associated with specific long-term eating patterns
- The phylogenetic profile of each individual can be categorized into 3 enterotypes dominated by different metabolic pathways

  Enterotype 1 – *Bacterioides*

  Enterotype 2 – *Prevotella*

  Enterotype 3 – *Ruminococcus*

- Age, gender and body mass don't appear to influence enterotype

Arumugam et al., Enterotypes of the human gut microbiome, 2011 *Nature* **473:** 174–180

High fiber Diet

Bacteroidetes

↑ *Bacteroides-Prevotella* spp.

Firmicutes

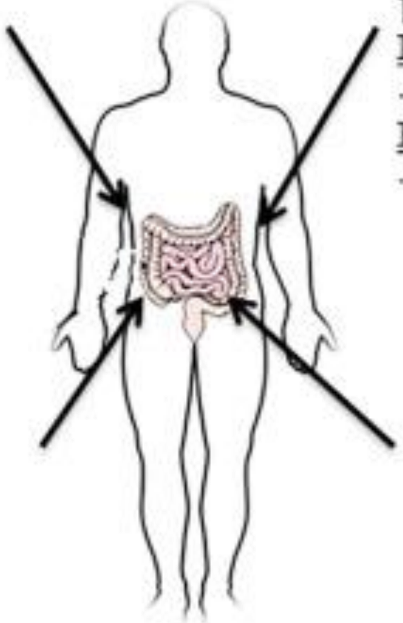
↑ *Bacilli* ↑*Erysipelotrichi* ↑ *Roseburia* spp.
↑ *Clostridia* group
↑ *Ruminococcus* ↑*Lactobacillus-Enterococcus* group ↑ *F. prausnitzii* and *E. rectale-C. coccoides* groups

Actinobacteria

Bifidobacterium ↑

Proteobacteria

↓ *Desulfovibrio*



Bacteroidetes

↑ *Bacteroides* enterotype
↓*Prevotella* enterotype
↑*Lactobacillus*
↑*Bifidobacterium*
↑ *Rikenella*
↑ *Peptostreptococcus*
↑ *Bacteroides* enterotype

Proteobacteria

↑ *Desulfovibrio*

Deferribacteres

-*Mucisprillum* ↑

Firmicutes

- *Clostridium genus* ↑

High protein diet

High fat diet

Bacteroidetes↓

Rikenellaceae ↓ *Bacteroides* spp. ↓
↑*Bacteroides* enterotype

Firmicutes

Eubacterium rectale ↓ *Blautia coccoides* ↓ *Ruminococcaceae* ↓
Bacilli ↑
Erysipelotrichi ↑
Roseburia spp. ↑

Proteobacteria

Desulfovibrio ↓

Bacteroidetes

↑ *Prevotella* enterotype

Firmicutes

↑ *Roseburia* groups
↑ *Bifidobacteria*
↑ *E. rectale*
↑*Ruminococcus bromii*
↑ *R. flavefaciens* ↑ *R. albus*

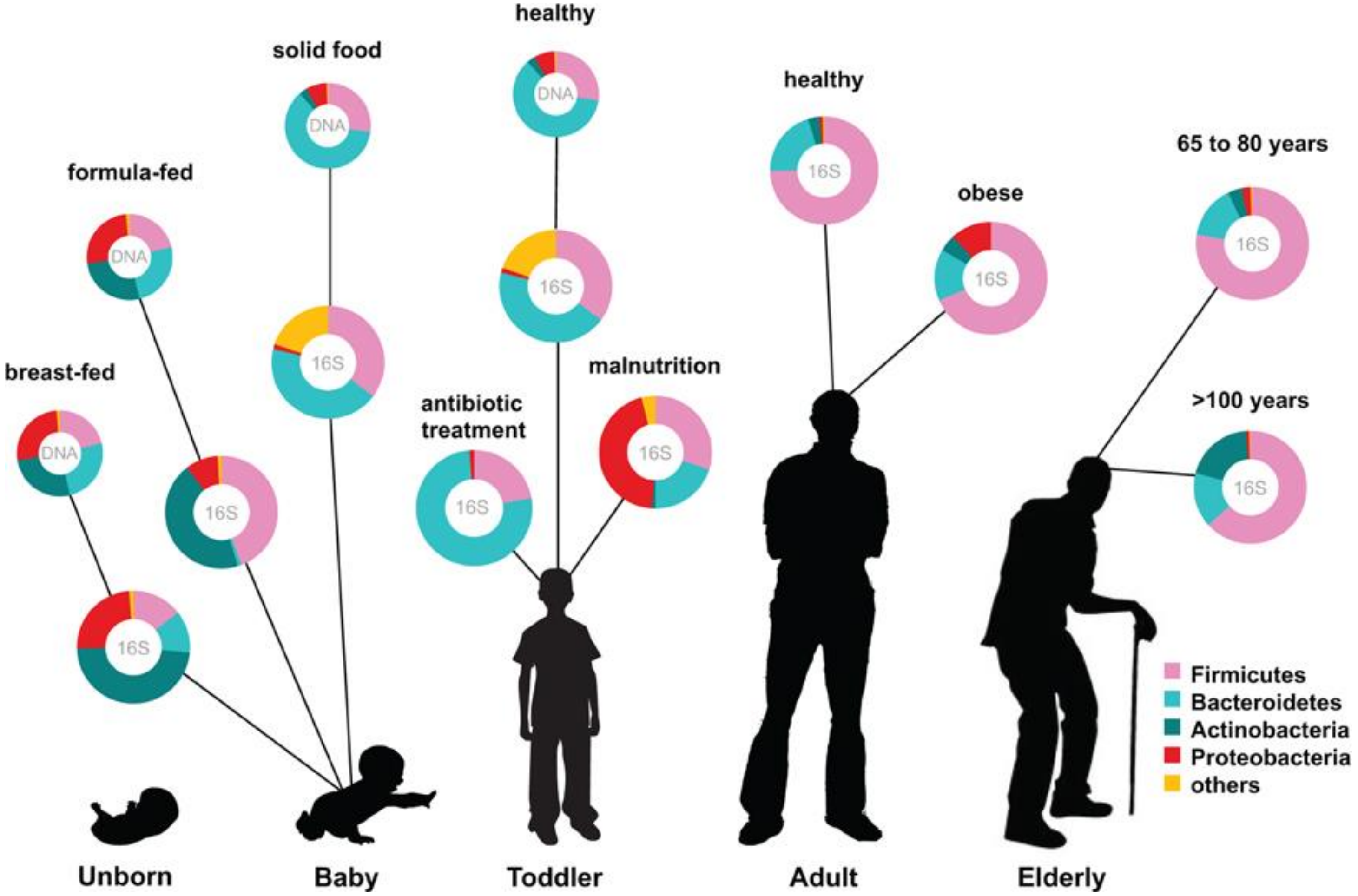
Actinobacteria

↑ *Bifidobacterium*

Carbohydrates diet

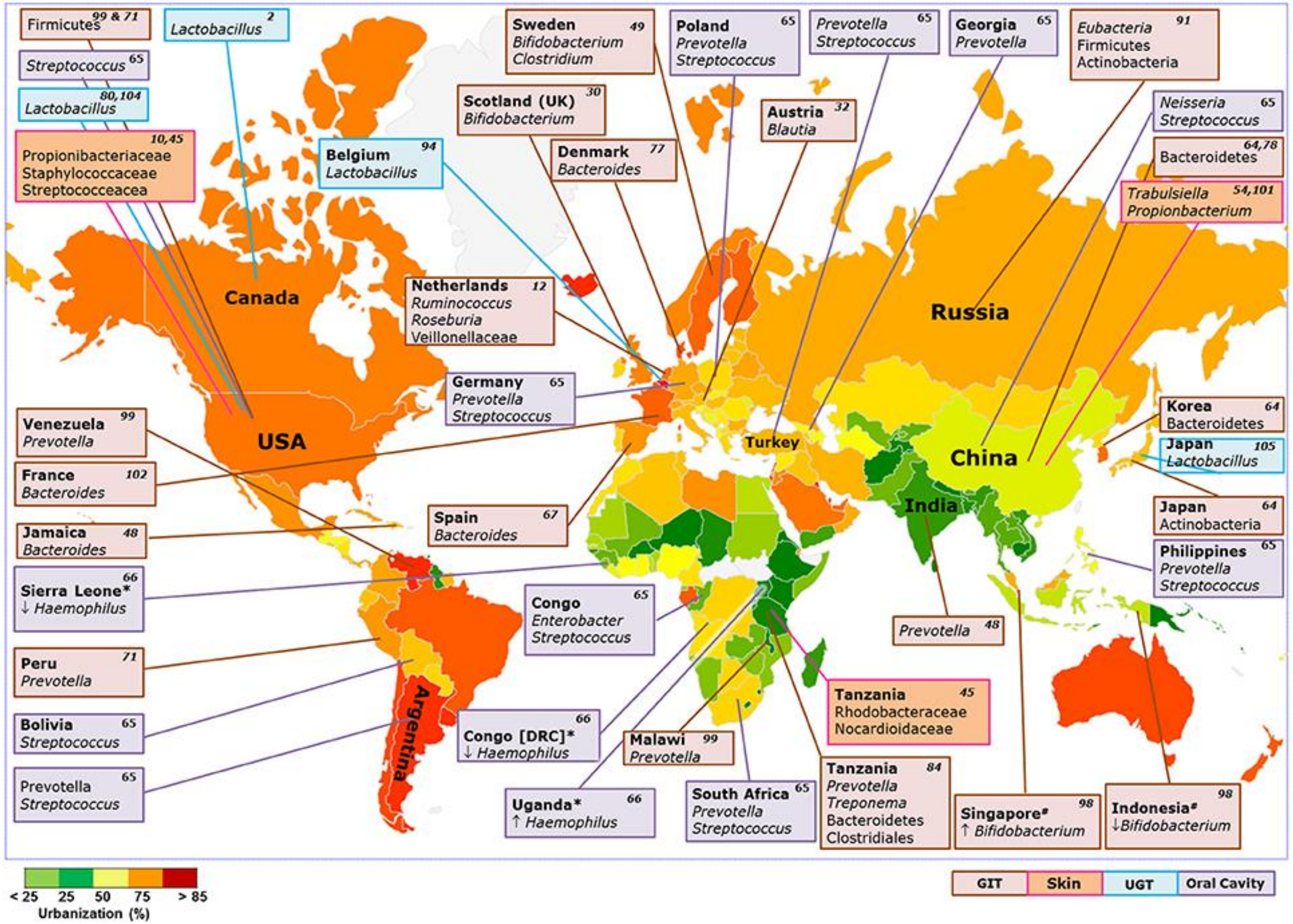
Ageing

- **onset and shaping of human gut microbiota through life stages and perturbations**
- babies have low diversity of the microbiota
- the microbiota of 2.5 year olds is already similar to that of adults
- the microbiota of adults is stable
- with the age the diversity of microbiota declines (↓ diversity and metabolic activity – SCFA, ↓ immune system)



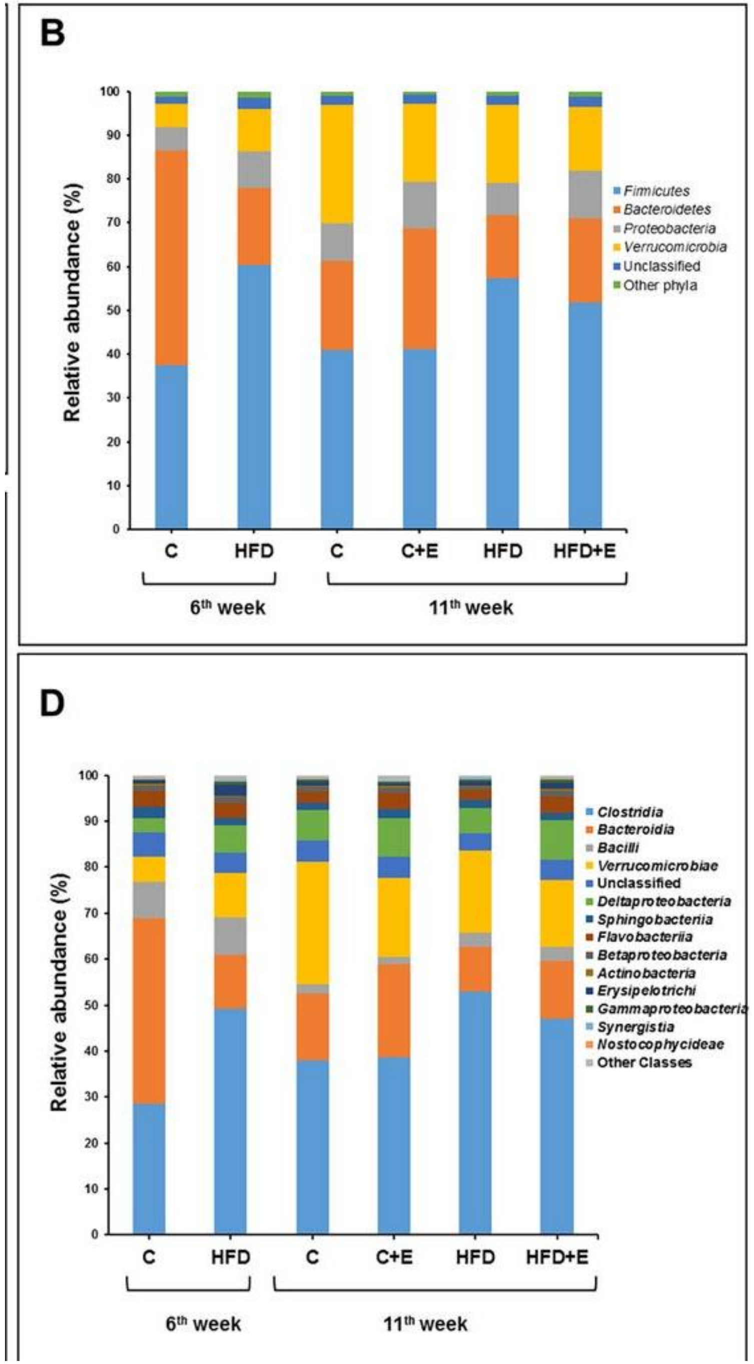
Geography

- The taxonomic composition of the gut microbiome associates with patient ethnicity and geographic location.
- Certain taxonomic groups of bacteria are a characteristic feature of a given geographical area irrespective of the diet or age of the population



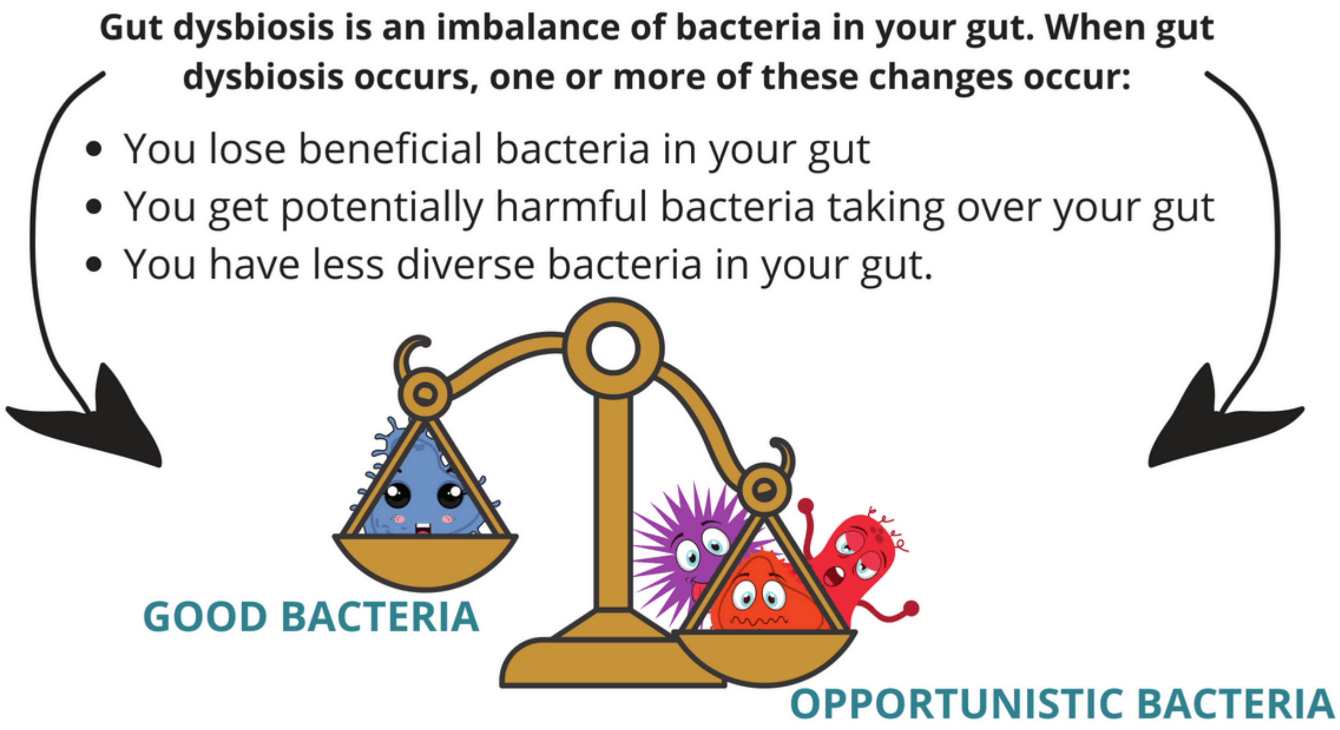
Exercise

- Physical exercise is able to modulate gut microbiota and increase the abudance of beneficial microbial species.
- Increasing physical activity in obese animals lead to the changes in gut microbiota composition connected with weight lose and lipid metabolism modulation



Carbajo-Pescador et al. 2019, Disease Models & Mechanisms 12, dmm039206

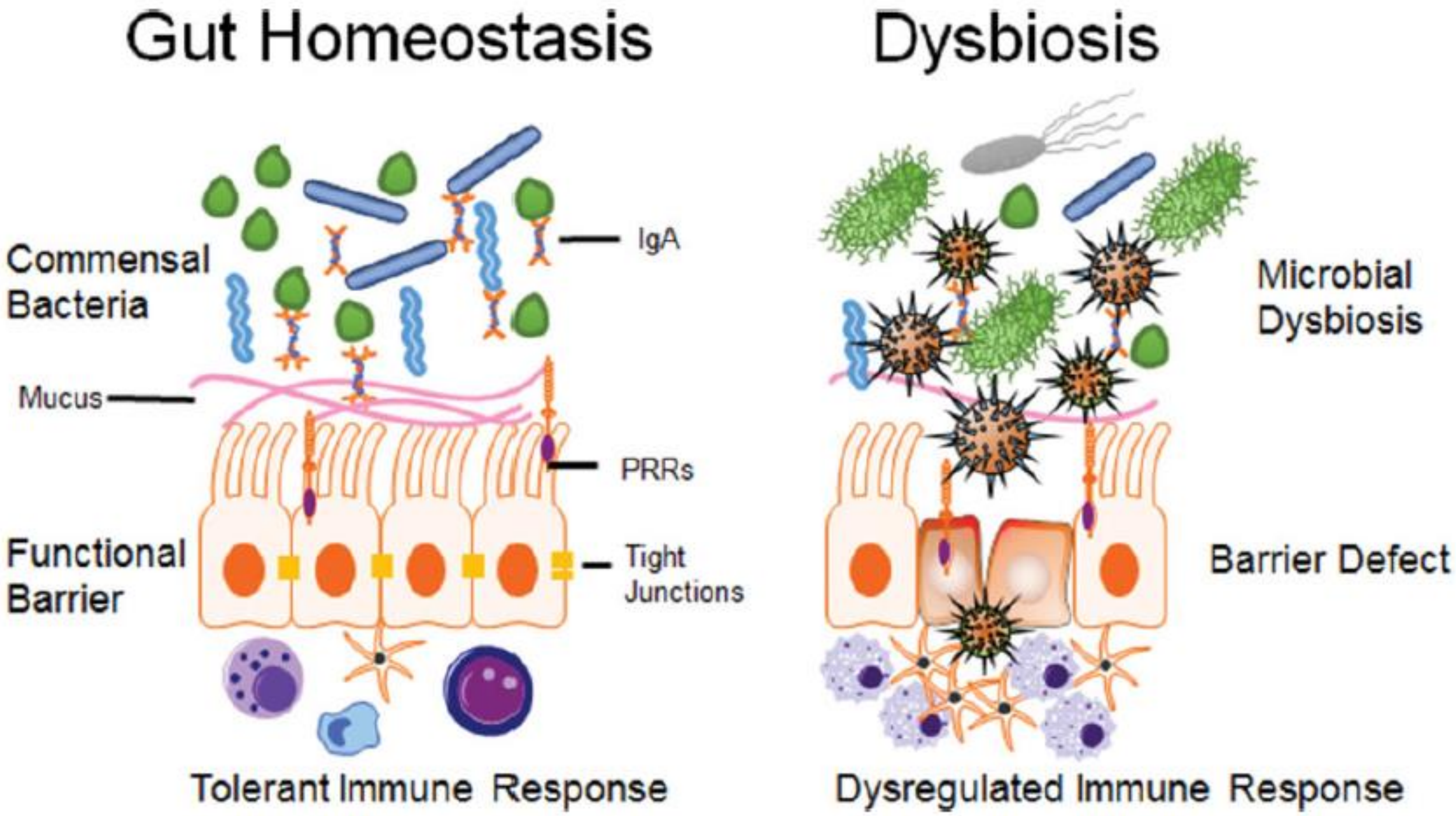
Dysbiosis



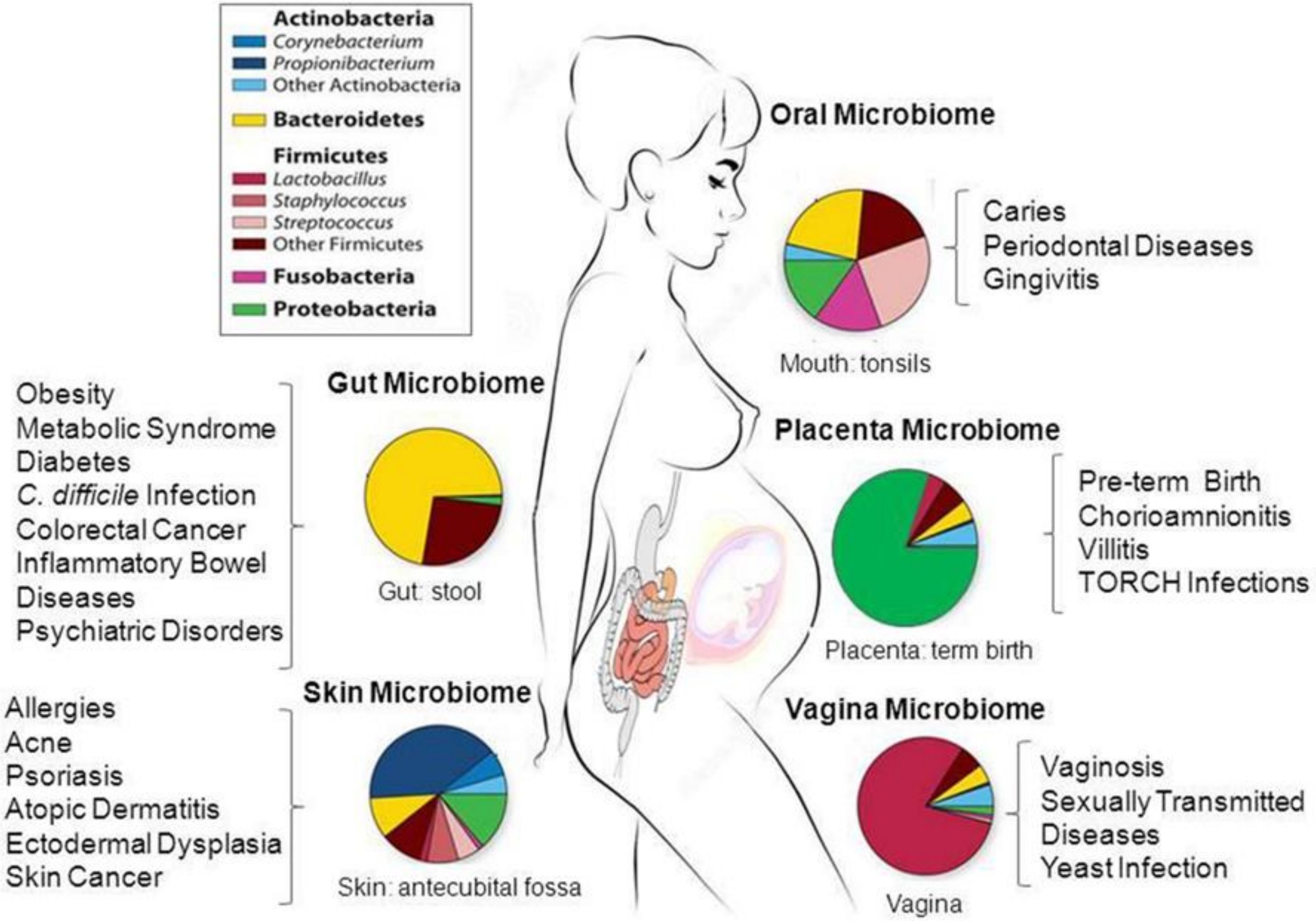
Gut dysbiosis is an imbalance of bacteria in your gut. When gut dysbiosis occurs, one or more of these changes occur:

- You lose beneficial bacteria in your gut
- You get potentially harmful bacteria taking over your gut
- You have less diverse bacteria in your gut.

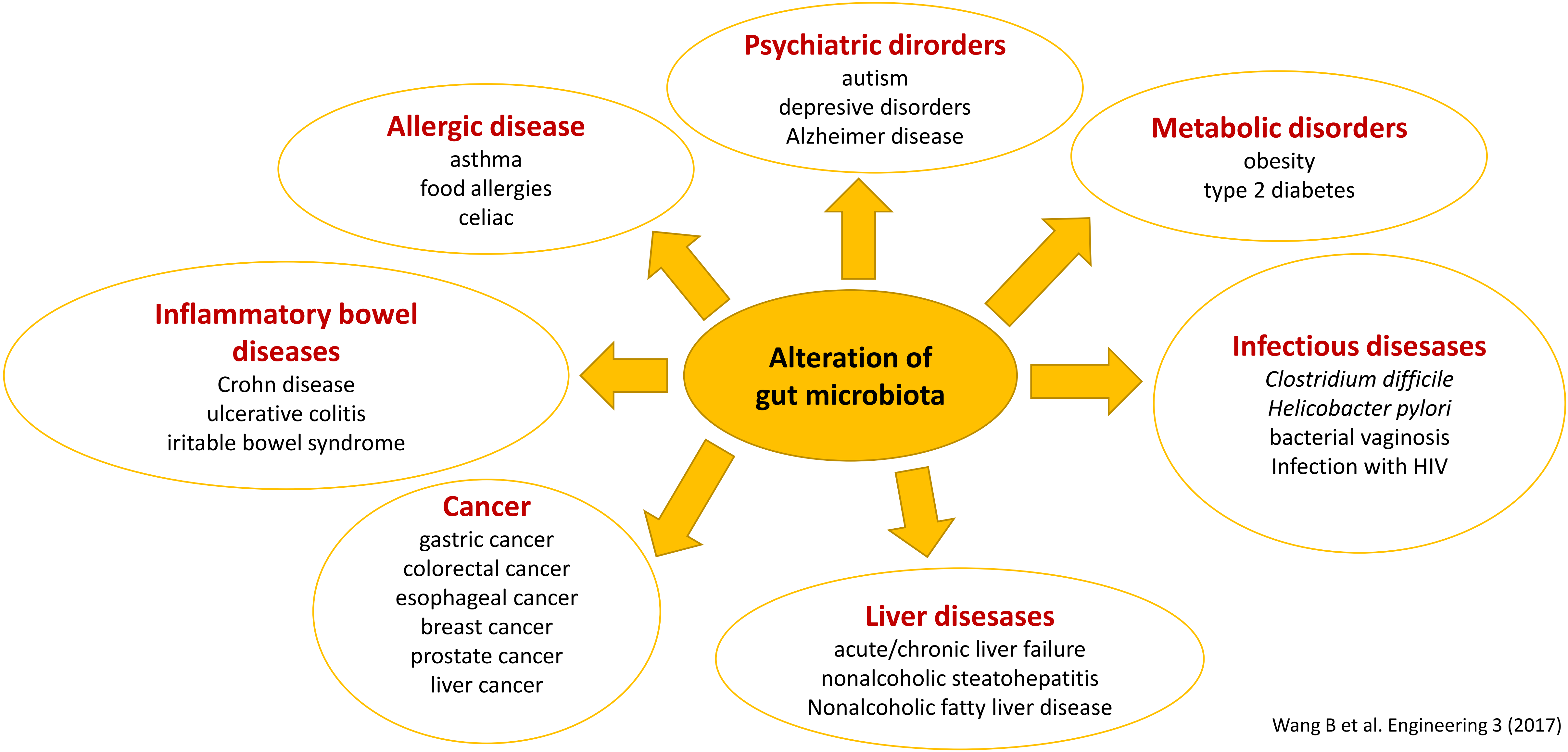
- Changes in the composition and functions of our microbiomes (**dysbiosis**) correlate with numerous **disease states**, raising the possibility that manipulation of these communities could be used to treat disease.


Gut Homeostasis
Dysbiosis
Commensal Bacteria
IgA
Mucus
PRRs
Functional Barrier
Tight Junctions
Tolerant Immune Response
Microbial Dysbiosis
Barrier Defect
Dysregulated Immune Response

Dysbiosis associated diseases



Gut dysbiosis-associated diseases



Alteration of gut microbiota

Psychiatric dirorders
autism
depresive disorders
Alzheimer disease

Allergic disease
asthma
food allergies
celiac

Metabolic disorders
obesity
type 2 diabetes

Inflammatory bowel diseases
Crohn disease
ulcerative colitis
iritable bowel syndrome

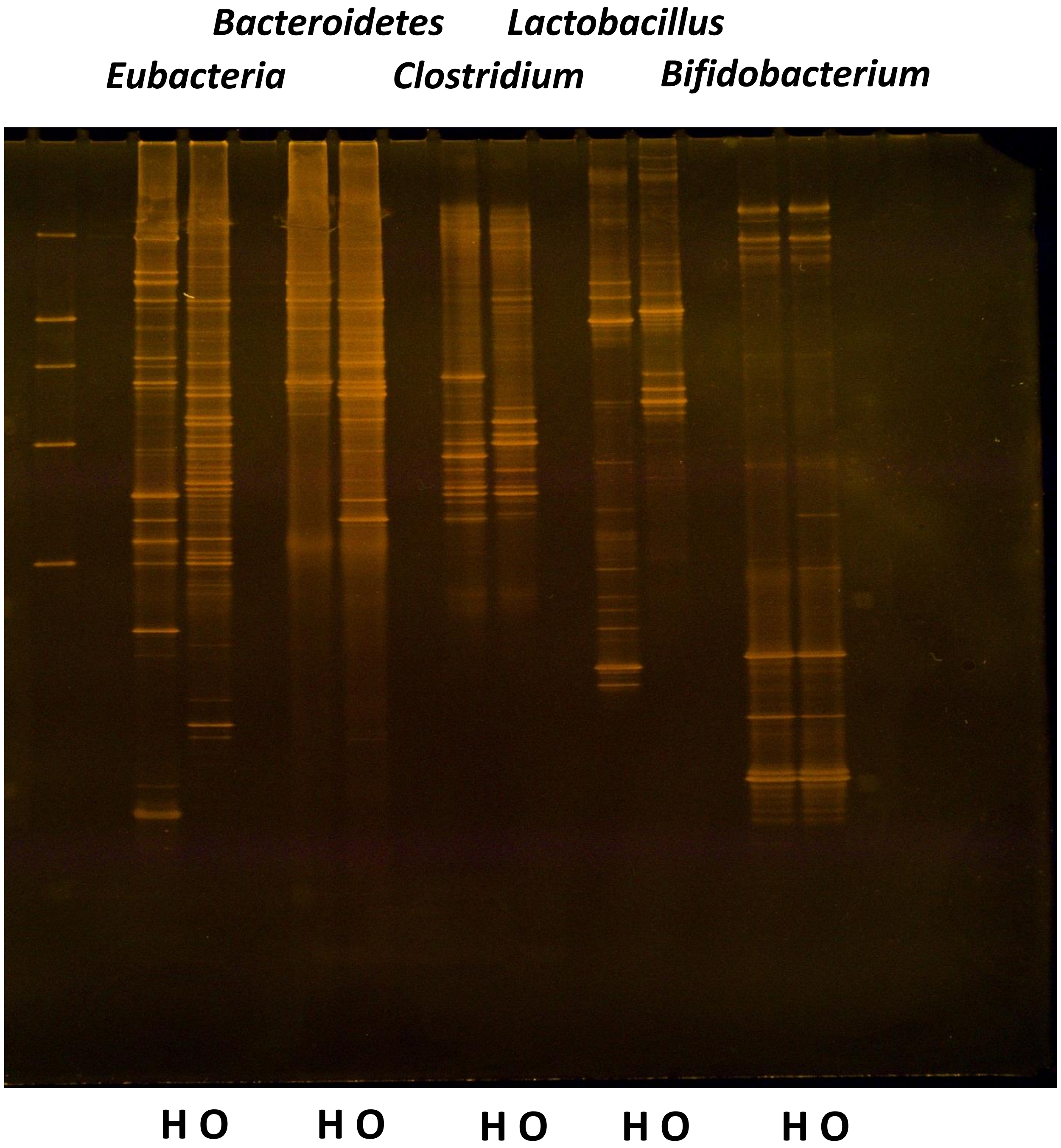
Infectious diseases
Clostridium difficile
Helicobacter pylori
bacterial vaginosis
Infection with HIV

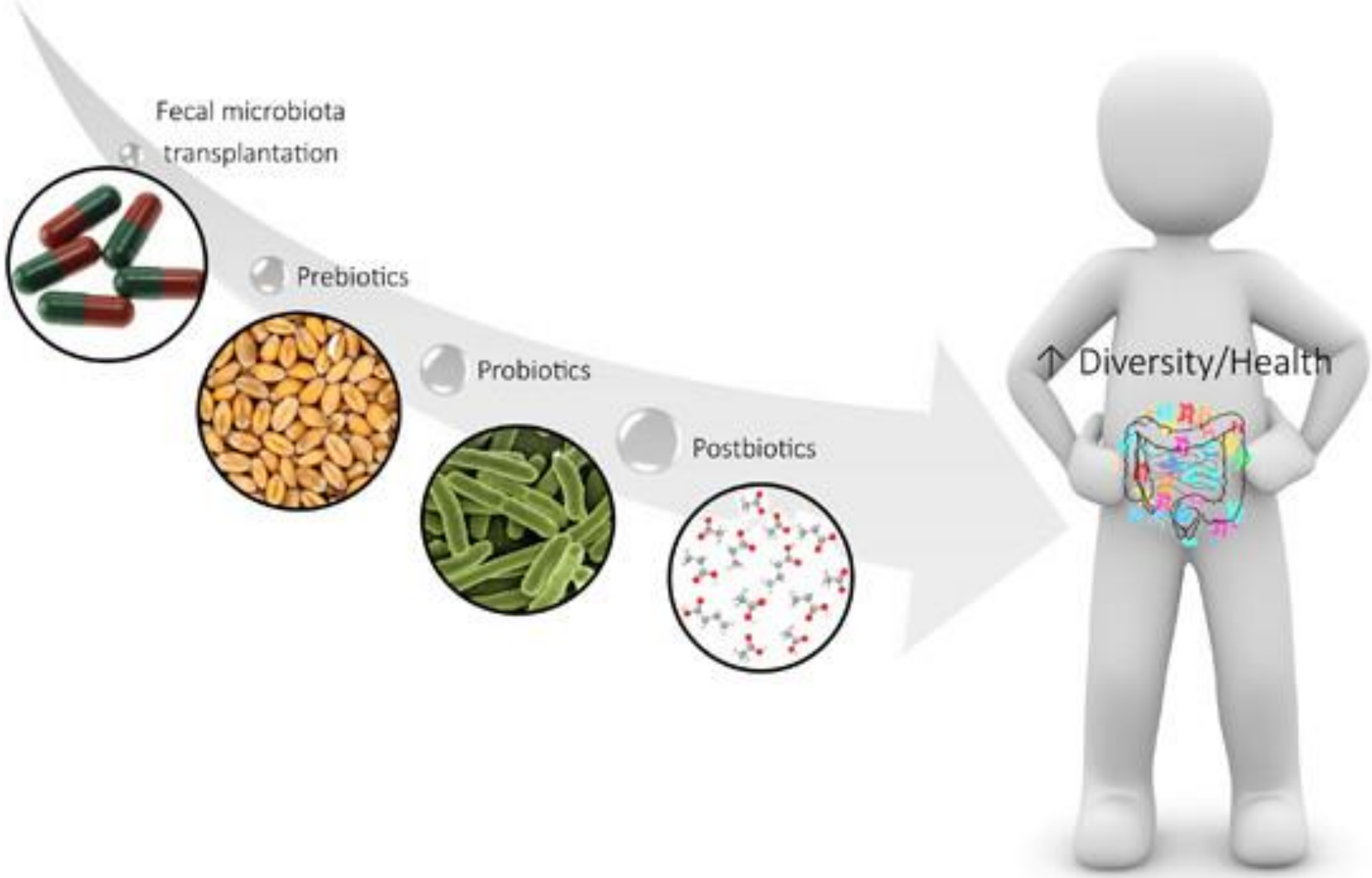
Cancer
gastric cancer
colorectal cancer
esophageal cancer
breast cancer
prostate cancer
liver cancer

Liver diseases
acute/chronic liver failure
nonalcoholic steatohepatitis
Nonalcoholic fatty liver disease

Wang B et al. Engineering 3 (2017) 71–82

Gut microbiota of healthy, lean vs. obese human





Fecal microbiota transplantation

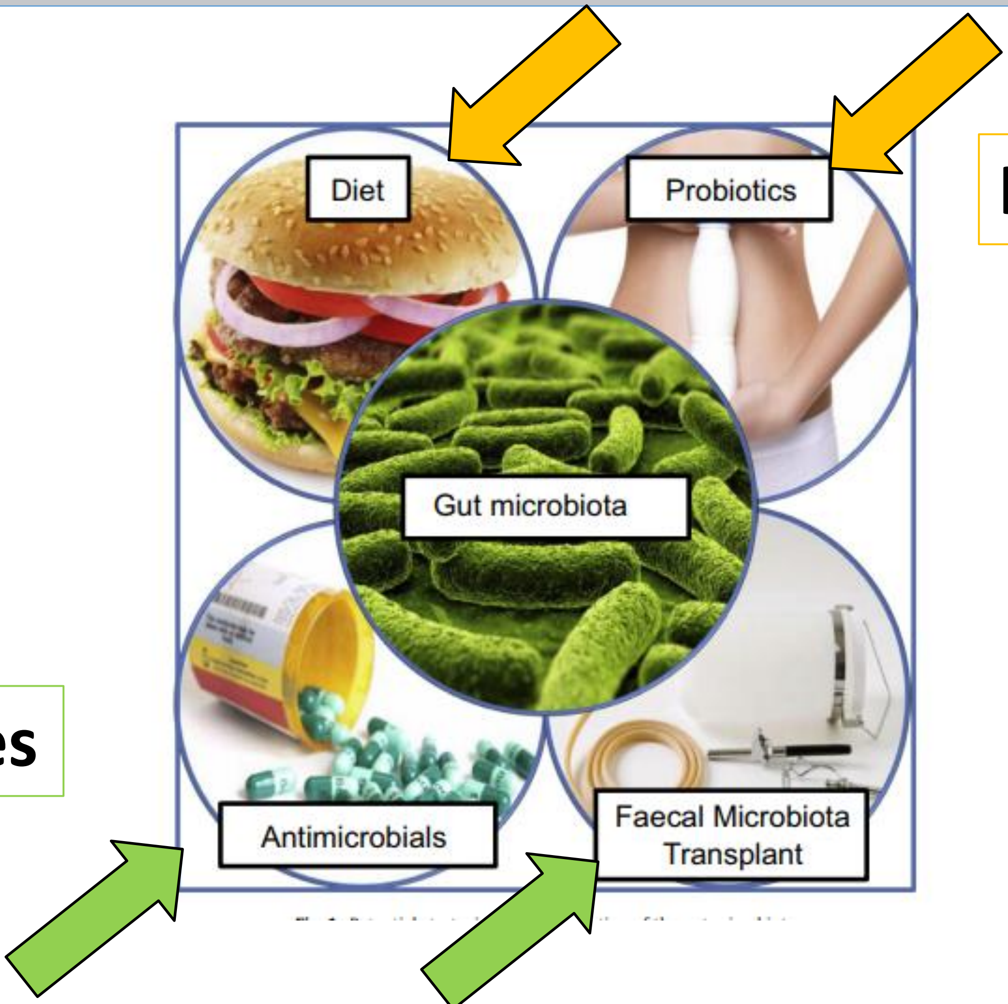
Prebiotics

Probiotics

Postbiotics

↑ Diversity/Health

Manipulation of gut microbiome



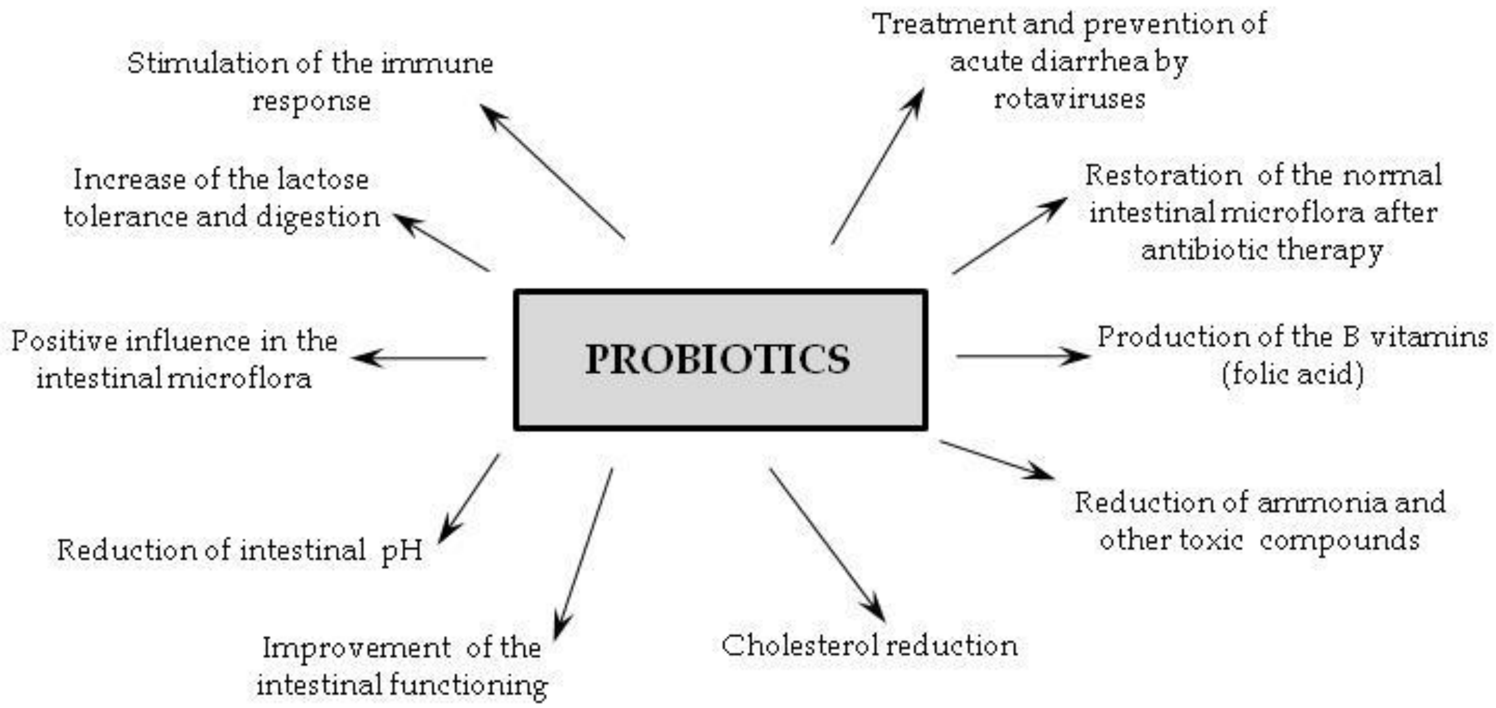
Indirect strategies

Direct strategies

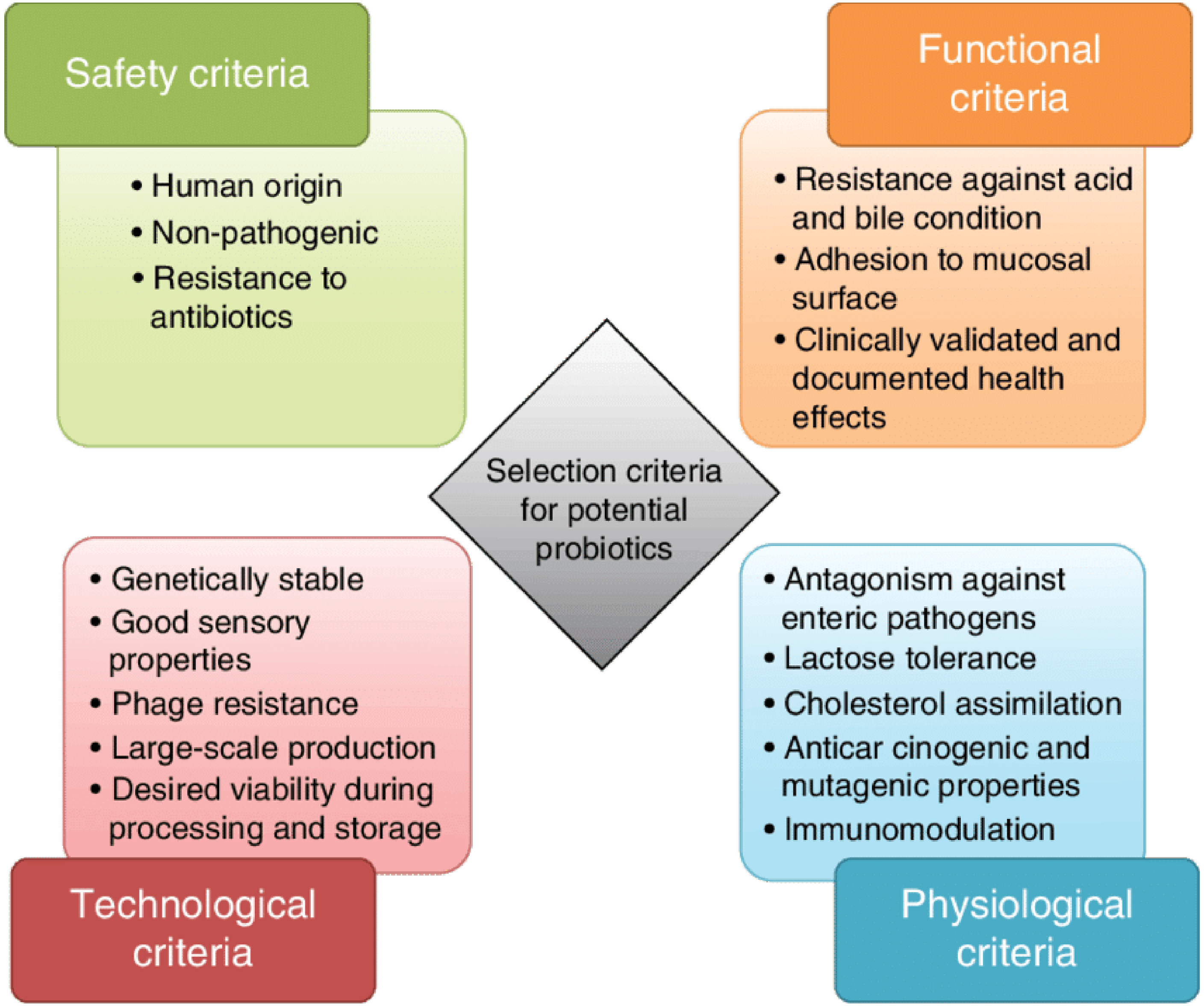
C.J. Walsh et al. FEBS Letters 588 (2014) 4120–4130

Probiotics

- **Probiotics are defined as living bacteria that, when administered in adequate amounts, confer a health benefit on the host (FAO/ WHO 2001).**



Desirable selection criteria for potential probiotic microorganisms



Selection criteria for potential probiotics

Safety criteria

- Human origin
- Non-pathogenic
- Resistance to antibiotics

Functional criteria

- Resistance against acid and bile condition
- Adhesion to mucosal surface
- Clinically validated and documented health effects

Technological criteria

- Genetically stable
- Good sensory properties
- Phage resistance
- Large-scale production
- Desired viability during processing and storage

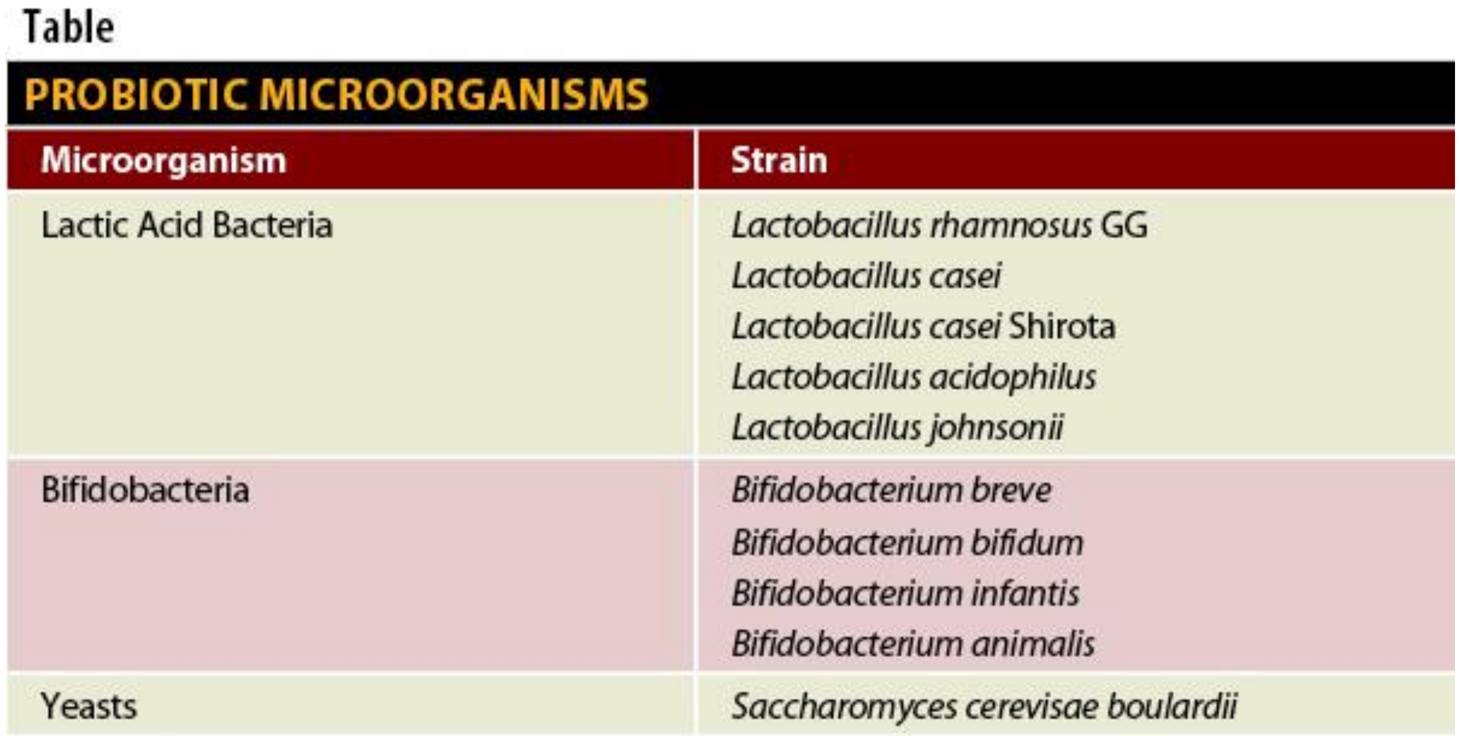
Physiological criteria

- Antagonism against enteric pathogens
- Lactose tolerance
- Cholesterol assimilation
- Anticar cinogenic and mutagenic properties
- Immunomodulation

Probiotics

- CONVENTIAL PROBIOTICS
- POTENTIATED PROBIOTICS and SYNBIOTICS
- ENGINEERED PROBIOTICS
- AUTOPROBIOTICS
- NEXT GENERATION PROBIOTICS

Conventional probiotics



Table

PROBIOTIC MICROORGANISMS

Microorganism	Strain
Lactic Acid Bacteria	*Lactobacillus rhamnosus* GG *Lactobacillus casei* *Lactobacillus casei* Shirota *Lactobacillus acidophilus* *Lactobacillus johnsonii*
Bifidobacteria	*Bifidobacterium breve* *Bifidobacterium bifidum* *Bifidobacterium infantis* *Bifidobacterium animalis*
Yeasts	*Saccharomyces cerevisae boulardii*

The effect is strain specific!!!

Prebiotic

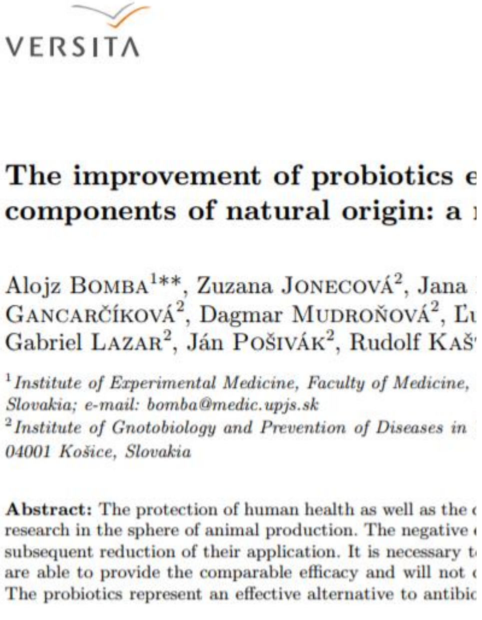
- a non-digestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host.

- **Synbiotic**

Potentiated probiotics

- **Improvement of the probiotic effect of microorganisms by their combination with specific and non-specific substrates = synbiotics**

- **Enhancement of the probiotic effect of microorganisms by their combination with plants,**



VERSITA

The improvement of probiotics e
components of natural origin: a

Alojz BOMBA[1**], Zuzana JONECOVÁ[2], Jana
GANCARČÍKOVÁ[2], Dagmar MUDROŇOVÁ[2], Ľu
Gabriel LAZAR[2], Ján POŠIVÁK[2], Rudolf KAŠ

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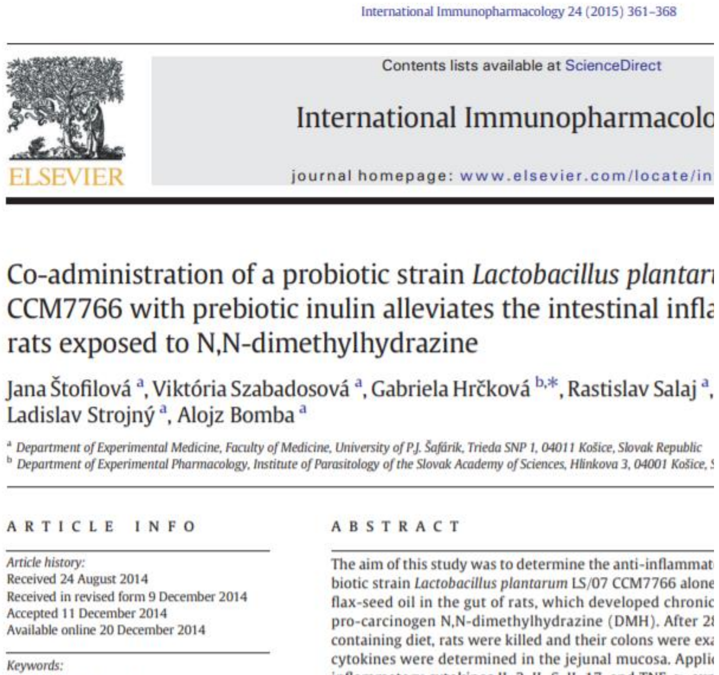
Abstract: The protection of human health as well as the
research in the sphere of animal production. The negative
subsequent reduction of their application. It is necessary t
are able to provide the comparable efficacy and will not
The probiotics represent an effective alternative to antibic



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ELSEVIER

Co-administration of a probiotic strain *Lactobacillus plantar*
CCM7766 with prebiotic inulin alleviates the intestinal infla
rats exposed to N,N-dimethylhydrazine

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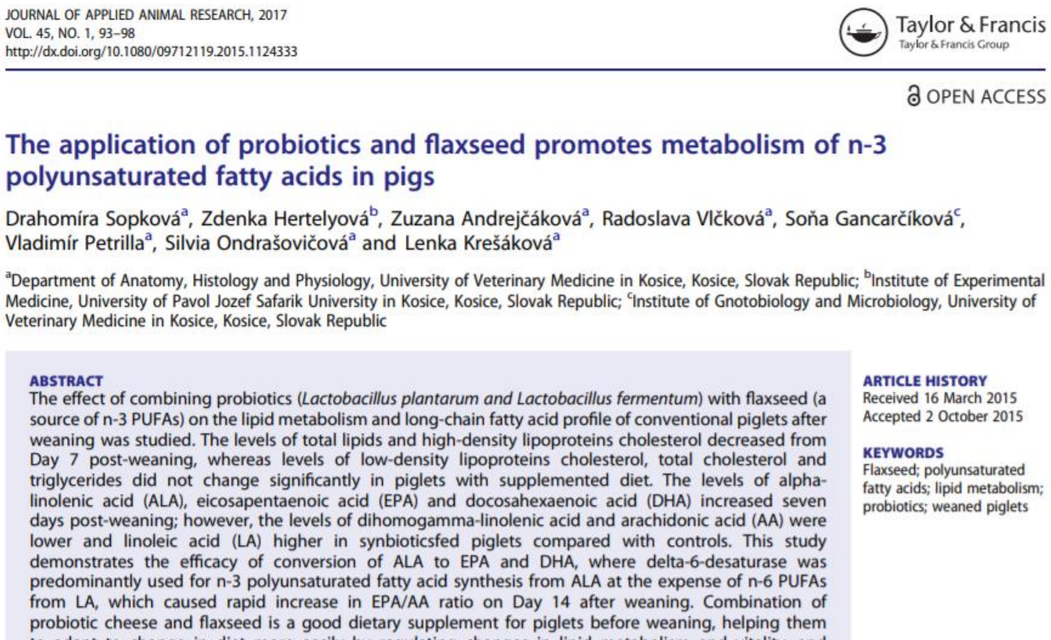
ABSTRACT

The aim of this study was to determine the anti-inflammat
biotic strain *Lactobacillus plantarum* LS/07 CCM7766 alone
flax-seed oil in the gut of rats, which developed chronic
pro-carcinogen N,N-dimethylhydrazine (DMH). After 2
containing diet, rats were killed and their colons were ex
cytokines were determined in the jejunal mucosa. Appli



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Taylor & Francis
Taylor & Francis Group

OPEN ACCESS

The application of probiotics and flaxseed promotes metabolism of n-3 polyunsaturated fatty acids in pigs

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ABSTRACT

The effect of combining probiotics (*Lactobacillus plantarum and Lactobacillus fermentum*) with flaxseed (a source of n-3 PUFAs) on the lipid metabolism and long-chain fatty acid profile of conventional piglets after weaning was studied. The levels of total lipids and high-density lipoproteins cholesterol decreased from Day 7 post-weaning, whereas levels of low-density lipoproteins cholesterol, total cholesterol and triglycerides did not change significantly in piglets with supplemented diet. The levels of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased seven days post-weaning; however, the levels of dihomogamma-linolenic acid and arachidonic acid (AA) were lower and linoleic acid (LA) higher in synbioticsfed piglets compared with controls. This study demonstrates the efficacy of conversion of ALA to EPA and DHA, where delta-6-desaturase was predominantly used for n-3 polyunsaturated fatty acid synthesis from ALA at the expense of n-6 PUFAs from LA, which caused rapid increase in EPA/AA ratio on Day 14 after weaning. Combination of probiotic cheese and flaxseed is a good dietary supplement for piglets before weaning, helping them

ARTICLE HISTORY
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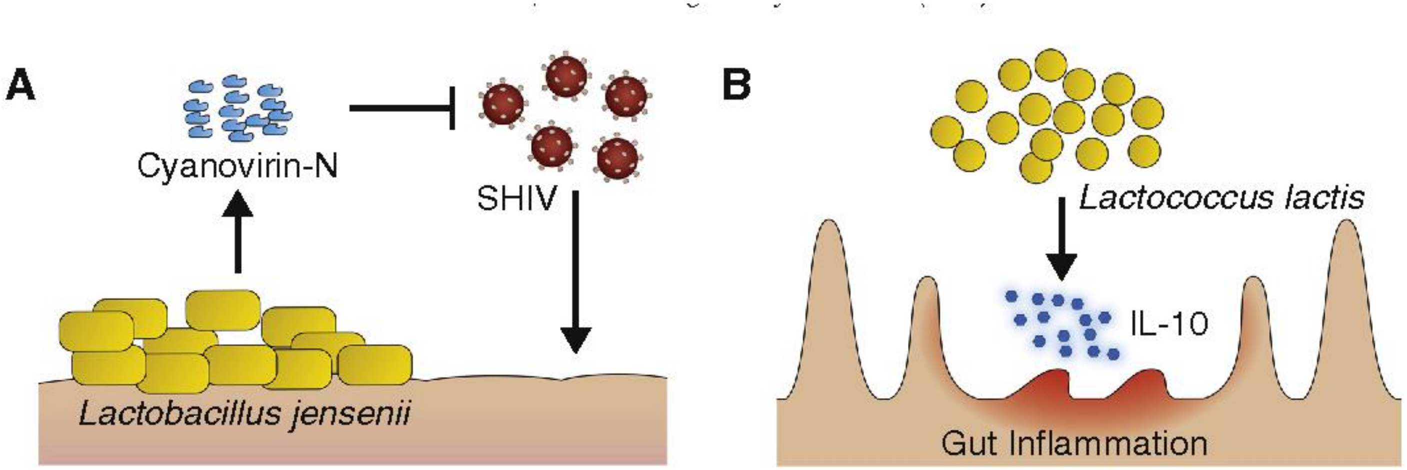
KEYWORDS
Flaxseed; polyunsaturated fatty acids; lipid metabolism; probiotics; weaned piglets

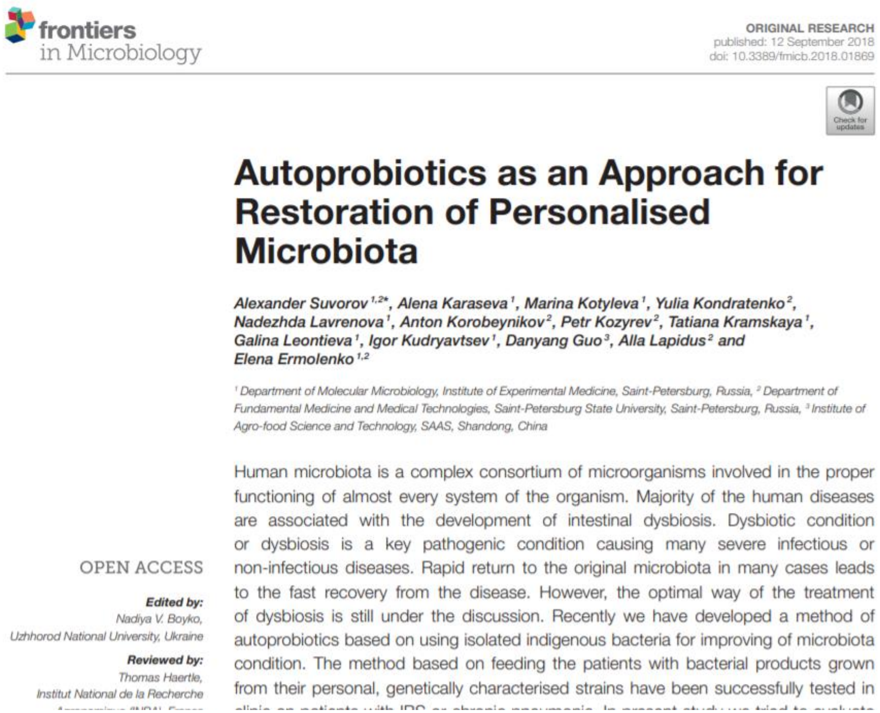
Engineered probiotics



- improve stress tolerance
- antimicrobial and antiviral action
- toxin neutralization
- prevention of colonization
- regulation of virulence gene expression
- production of antimicrobial factors
- immunomodulation and cytoprotection

Autoprobiotics

- Autoprobiotic technology is based on the indigenous bacteria used for restoring the normal microbiota in the case of a dysbiotic condition



frontiers
in Microbiology

ORIGINAL RESEARCH
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Autoprobiotics as an Approach for Restoration of Personalised Microbiota

Alexander Suvorov [1,2]*, Alena Karaseva [1], Marina Kotyleva [1], Yulia Kondratenko [2], Nadezhda Lavrenova [1], Anton Korobeynikov [2], Petr Kozyrev [2], Tatiana Kramskaya [1], Galina Leontieva [1], Igor Kudryavtsev [1], Danyang Guo [3], Alla Lapidus [2] and Elena Ermolenko [1,2]

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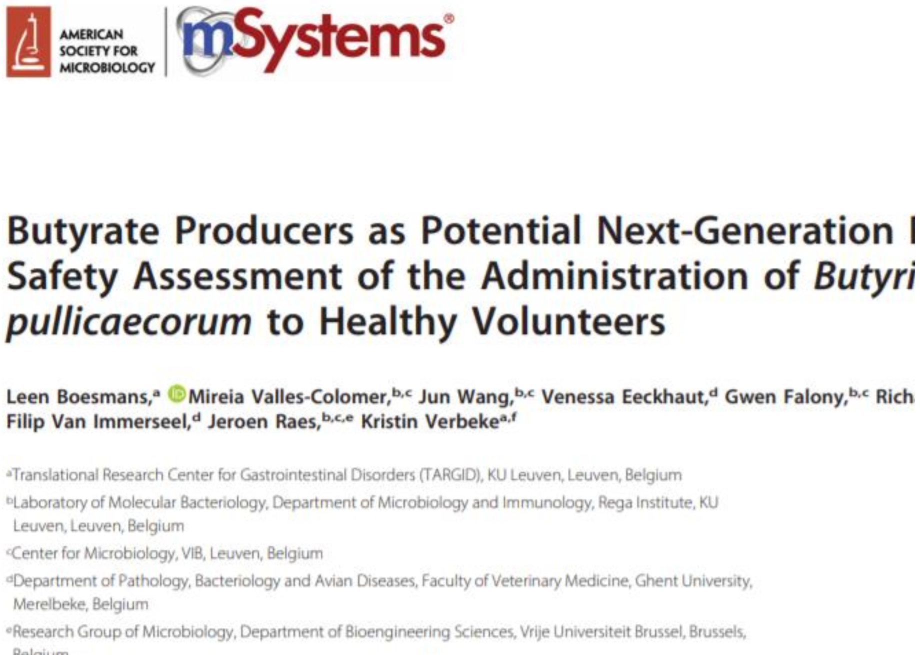
Institut National de la Recherche

Human microbiota is a complex consortium of microorganisms involved in the proper functioning of almost every system of the organism. Majority of the human diseases are associated with the development of intestinal dysbiosis. Dysbiotic condition or dysbiosis is a key pathogenic condition causing many severe infectious or non-infectious diseases. Rapid return to the original microbiota in many cases leads to the fast recovery from the disease. However, the optimal way of the treatment of dysbiosis is still under the discussion. Recently we have developed a method of autoprobiotics based on using isolated indigenous bacteria for improving of microbiota condition. The method based on feeding the patients with bacterial products grown from their personal, genetically characterised strains have been successfully tested in

Next generation probiotics

- Gut microbiota is a source of novel health-promoting bacteria, often termed as next-generation probiotics in order to distinguish them from traditional probiotics
- They do not have a long history of safe use and their safety is not thus considered as proven
- Live microorganisms identified on the basis of comparative microbiota analyses between both healthy and unhealthy individuals



AMERICAN SOCIETY FOR MICROBIOLOGY | mSystems®

Butyrate Producers as Potential Next-Generation I
Safety Assessment of the Administration of *Butyri*
***pullicaecorum* to Healthy Volunteers**

Leen Boesmans,[a] Mireia Valles-Colomer,[b,c] Jun Wang,[b,c] Venessa Eeckhaut,[d] Gwen Falony,[b,c] Rich
Filip Van Immerseel,[d] Jeroen Raes,[b,c,e] Kristin Verbeke[a,f]

[a]Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Leuven, Belgium
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[c]Center for Microbiology, VIB, Leuven, Belgium
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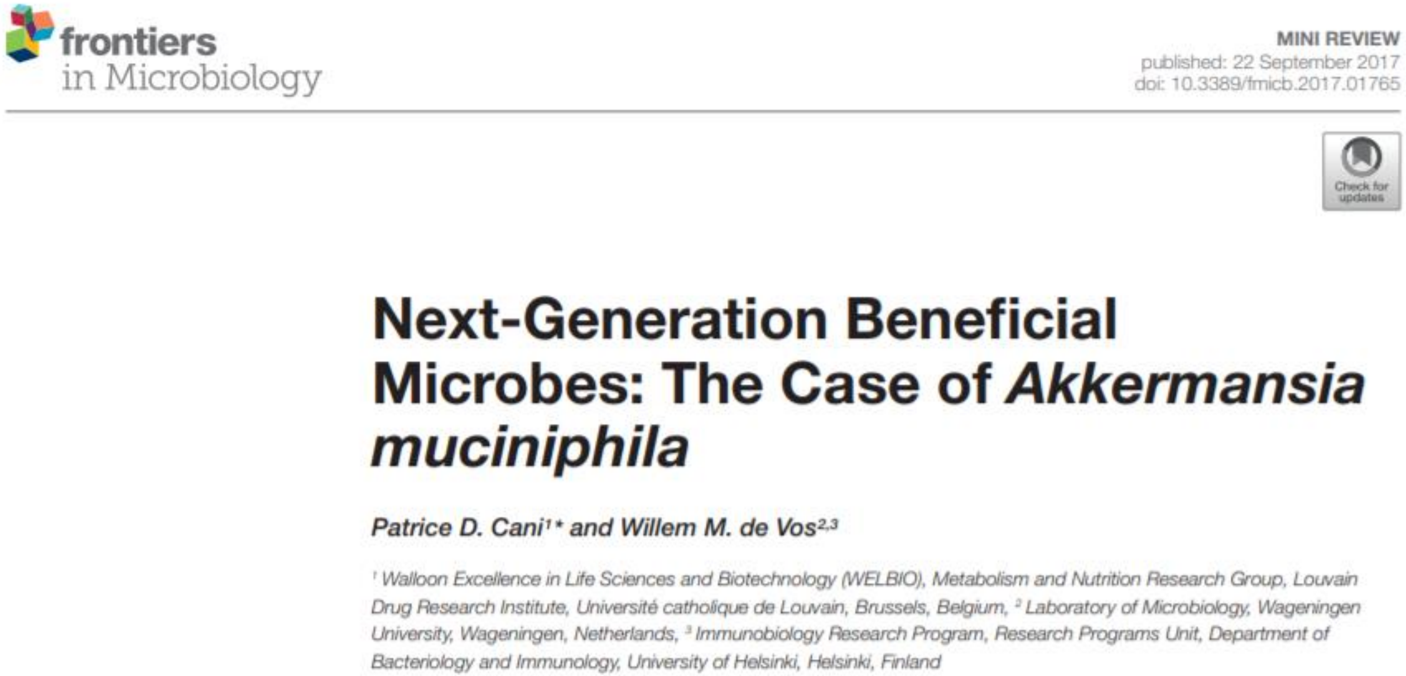


frontiers in Microbiology

MINI REVIEW
published: 22 September 2017
doi: 10.3389/fmicb.2017.01765

Check for updates

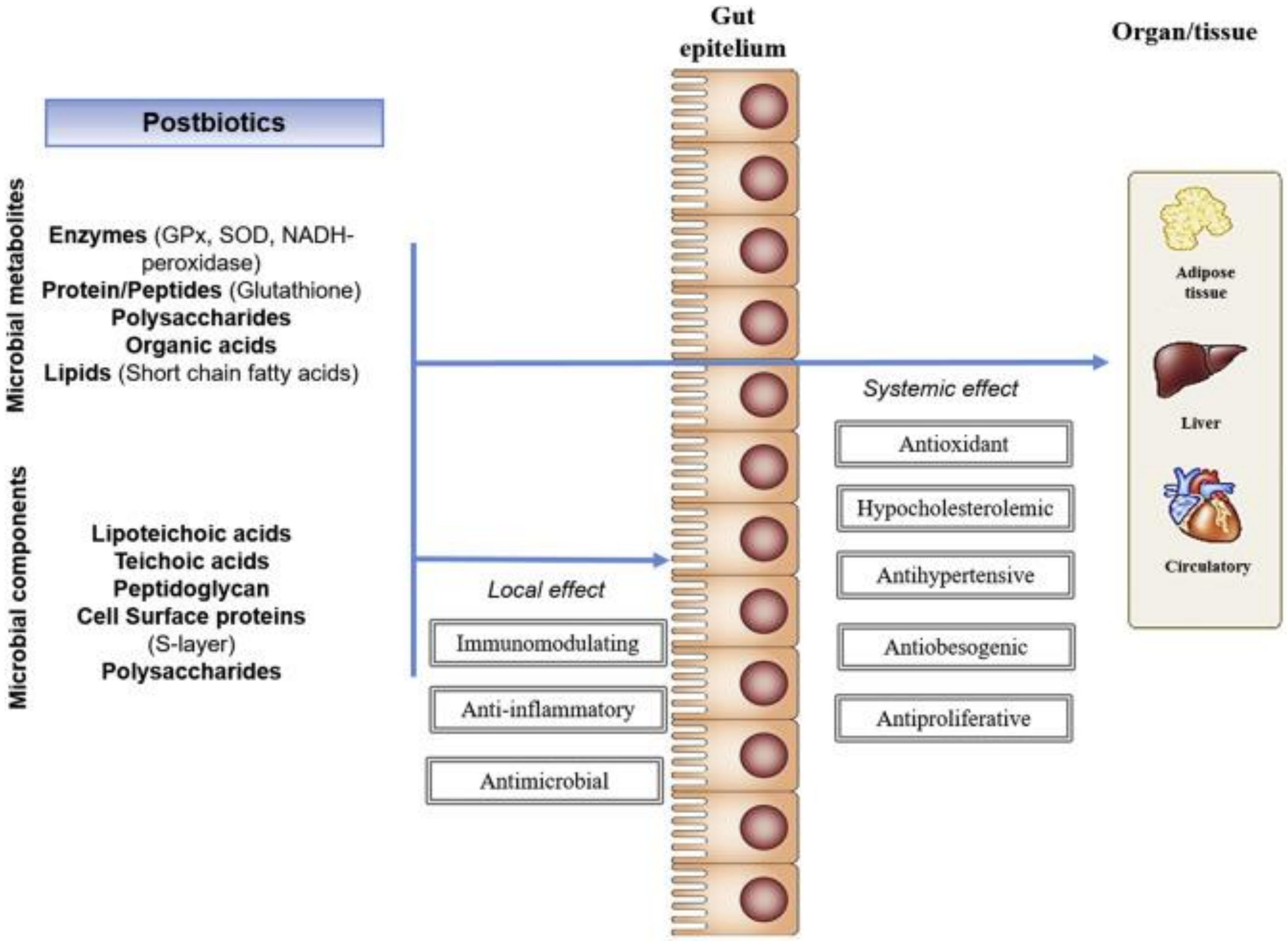
Next-Generation Beneficial Microbes: The Case of *Akkermansia muciniphila*

Patrice D. Cani[1]* and Willem M. de Vos[2,3]

[1] Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium, [2] Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands, [3] Immunobiology Research Program, Research Programs Unit, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland

Postbiotics

- **non-viable bacterial products or metabolic products from microorganisms that have biologic activity in the host**



Postbiotics

Microbial metabolites

Enzymes (GPx, SOD, NADH-peroxidase)
Protein/Peptides (Glutathione)
Polysaccharides
Organic acids
Lipids (Short chain fatty acids)

Microbial components

Lipoteichoic acids
Teichoic acids
Peptidoglycan
Cell Surface proteins
(S-layer)
Polysaccharides

Gut epitelium

Local effect

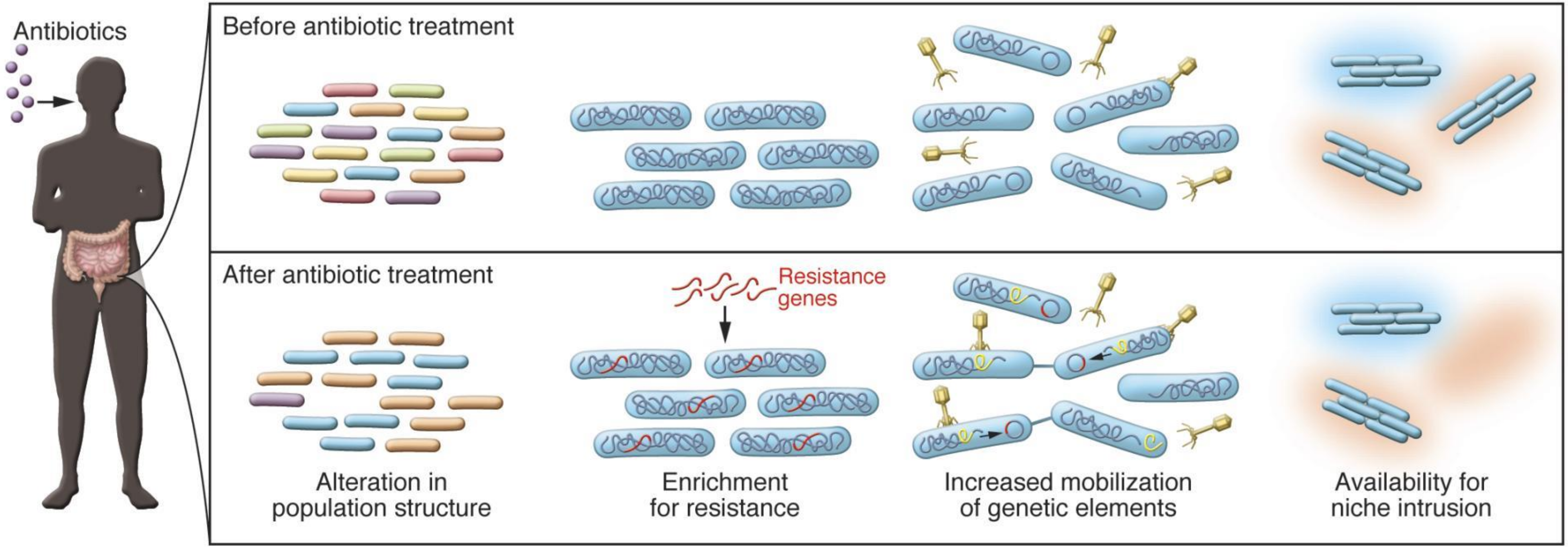
Immunomodulating
Anti-inflammatory
Antimicrobial

Systemic effect

Antioxidant
Hypocholesterolemic
Antihypertensive
Antiobesogenic
Antiproliferative

Organ/tissue

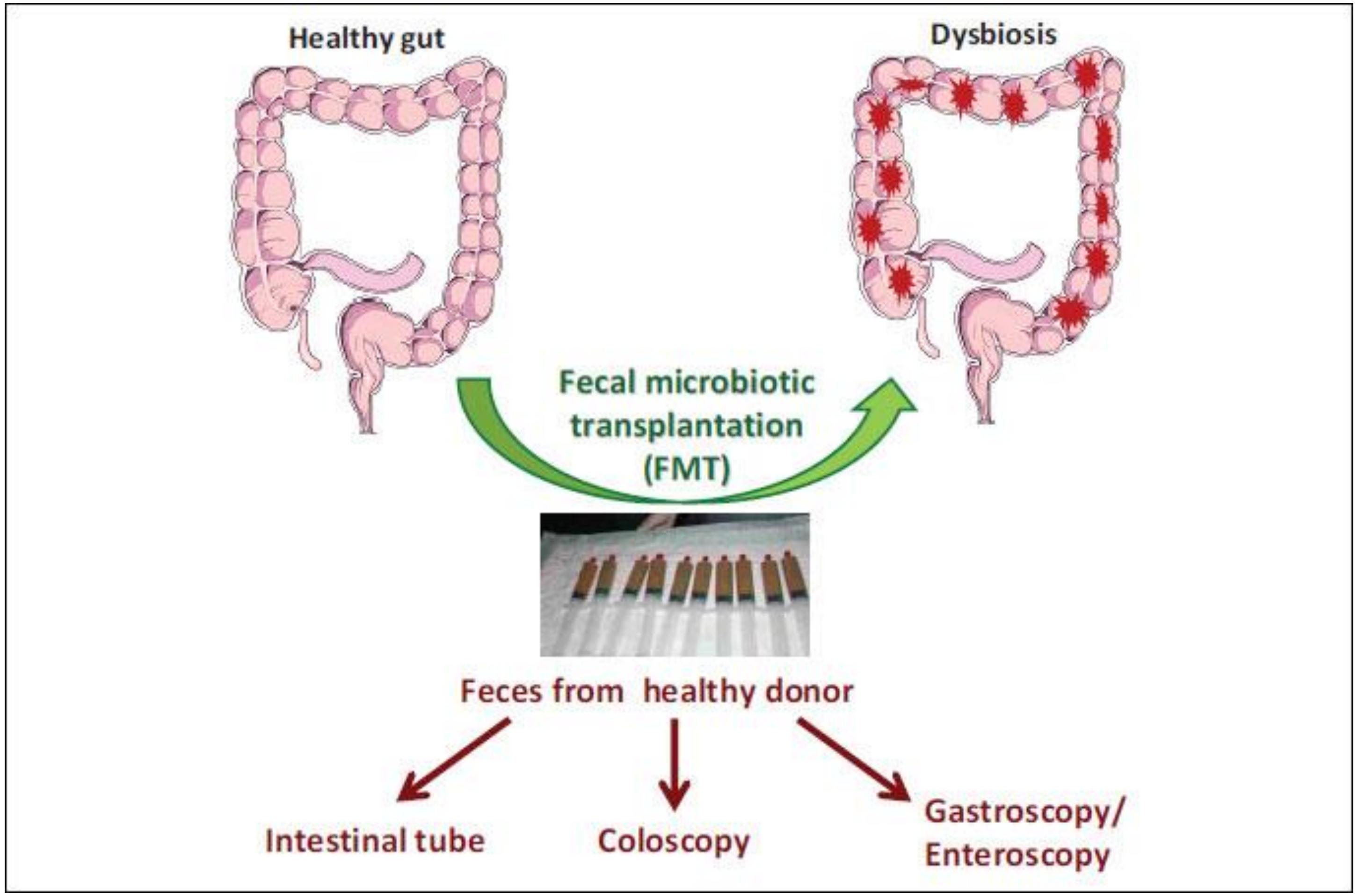
Antibiotics

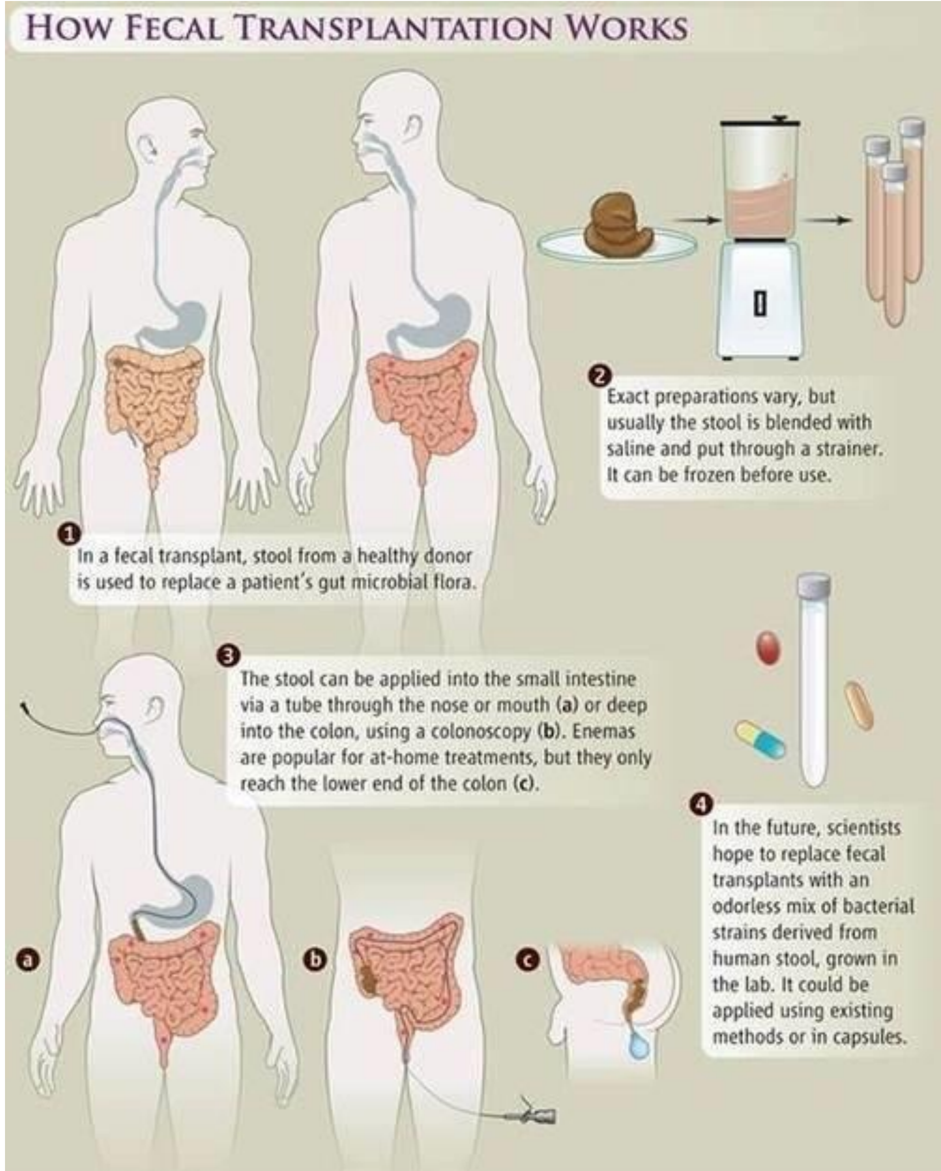


Antibiotic treatment alters the population structure of the indigenous microbiota, reducing bacterial diversity and redistributing member composition in both transient and persistent effects.

Fecal microbiota transplantation

- **FMT comprises the administration of a fecal solution from a donor into the intestinal tract of a recipient**





HOW FECAL TRANSPLANTATION WORKS

1 In a fecal transplant, stool from a healthy donor is used to replace a patient's gut microbial flora.

2 Exact preparations vary, but usually the stool is blended with saline and put through a strainer. It can be frozen before use.

3 The stool can be applied into the small intestine via a tube through the nose or mouth (**a**) or deep into the colon, using a colonoscopy (**b**). Enemas are popular for at-home treatments, but they only reach the lower end of the colon (**c**).

4 In the future, scientists hope to replace fecal transplants with an odorless mix of bacterial strains derived from human stool, grown in the lab. It could be applied using existing methods or in capsules.

Donor selection of fecal sample

- someone who is **healthy and on no medications**
- use same **exclusions as for blood** product **donation** (travel history, sexual behavior, previous operations, blood transfusions, etc...)
- screen donor for **a family history of autoimmune and metabolic diseases, malignancies**
- Screen blood and fecal sapmles for:

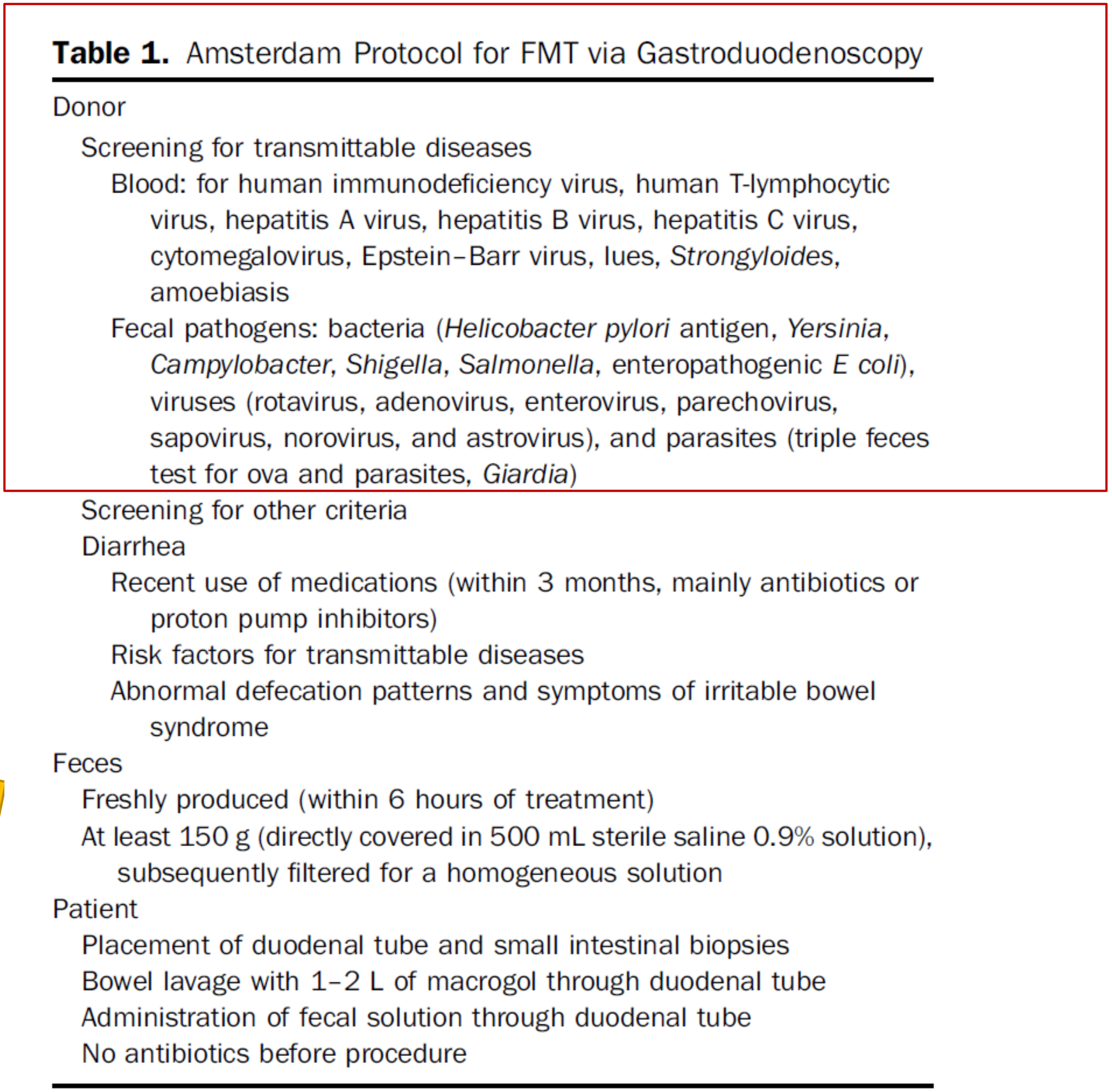


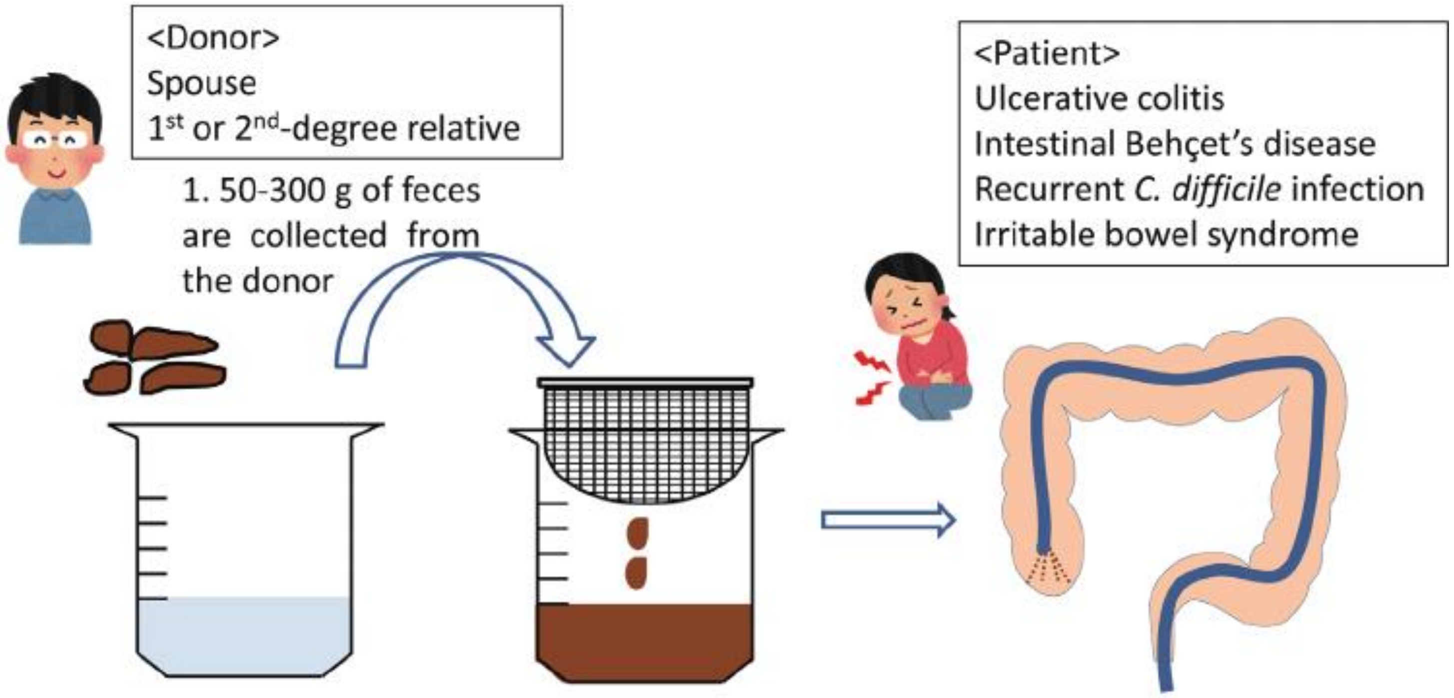
Table 1. Amsterdam Protocol for FMT via Gastroduodenoscopy

- Donor
  - Screening for transmittable diseases
    - Blood: for human immunodeficiency virus, human T-lymphocytic virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, cytomegalovirus, Epstein–Barr virus, lues, *Strongyloides*, amoebiasis
    - Fecal pathogens: bacteria (*Helicobacter pylori* antigen, *Yersinia*, *Campylobacter*, *Shigella*, *Salmonella*, enteropathogenic *E coli*), viruses (rotavirus, adenovirus, enterovirus, parechovirus, sapovirus, norovirus, and astrovirus), and parasites (triple feces test for ova and parasites, *Giardia*)
  - Screening for other criteria
  - Diarrhea
    - Recent use of medications (within 3 months, mainly antibiotics or proton pump inhibitors)
    - Risk factors for transmittable diseases
    - Abnormal defecation patterns and symptoms of irritable bowel syndrome
- Feces
  - Freshly produced (within 6 hours of treatment)
  - At least 150 g (directly covered in 500 mL sterile saline 0.9% solution), subsequently filtered for a homogeneous solution
- Patient
  - Placement of duodenal tube and small intestinal biopsies
  - Bowel lavage with 1–2 L of macrogol through duodenal tube
  - Administration of fecal solution through duodenal tube
  - No antibiotics before procedure

Smith LP et al. Gastroenterology 2013;145:946-953

Preparation of FMT material

- **Basic protocol:**



<Donor>
Spouse
1st or 2nd-degree relative

1. 50-300 g of feces are collected from the donor

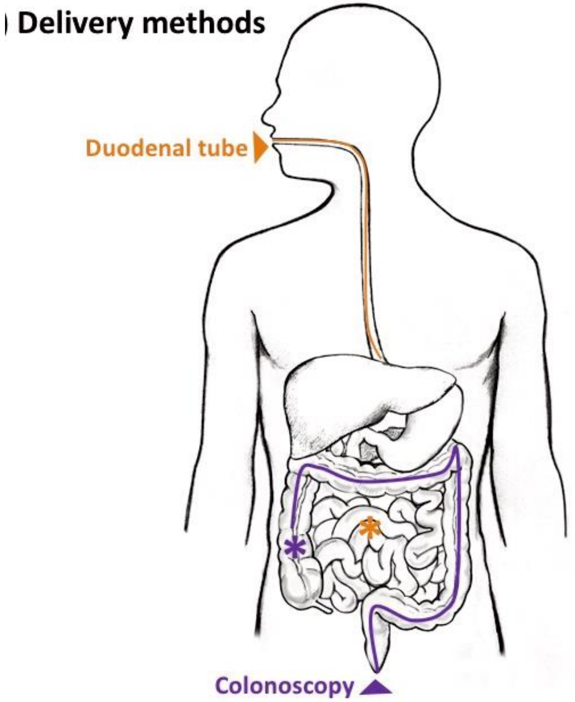
<Patient>
Ulcerative colitis
Intestinal Behçet's disease
Recurrent *C. difficile* infection
Irritable bowel syndrome

2. Feces are dissolved in 50-100 ml of normal saline.

3. Fecal materials are filtered through a metal strainer.

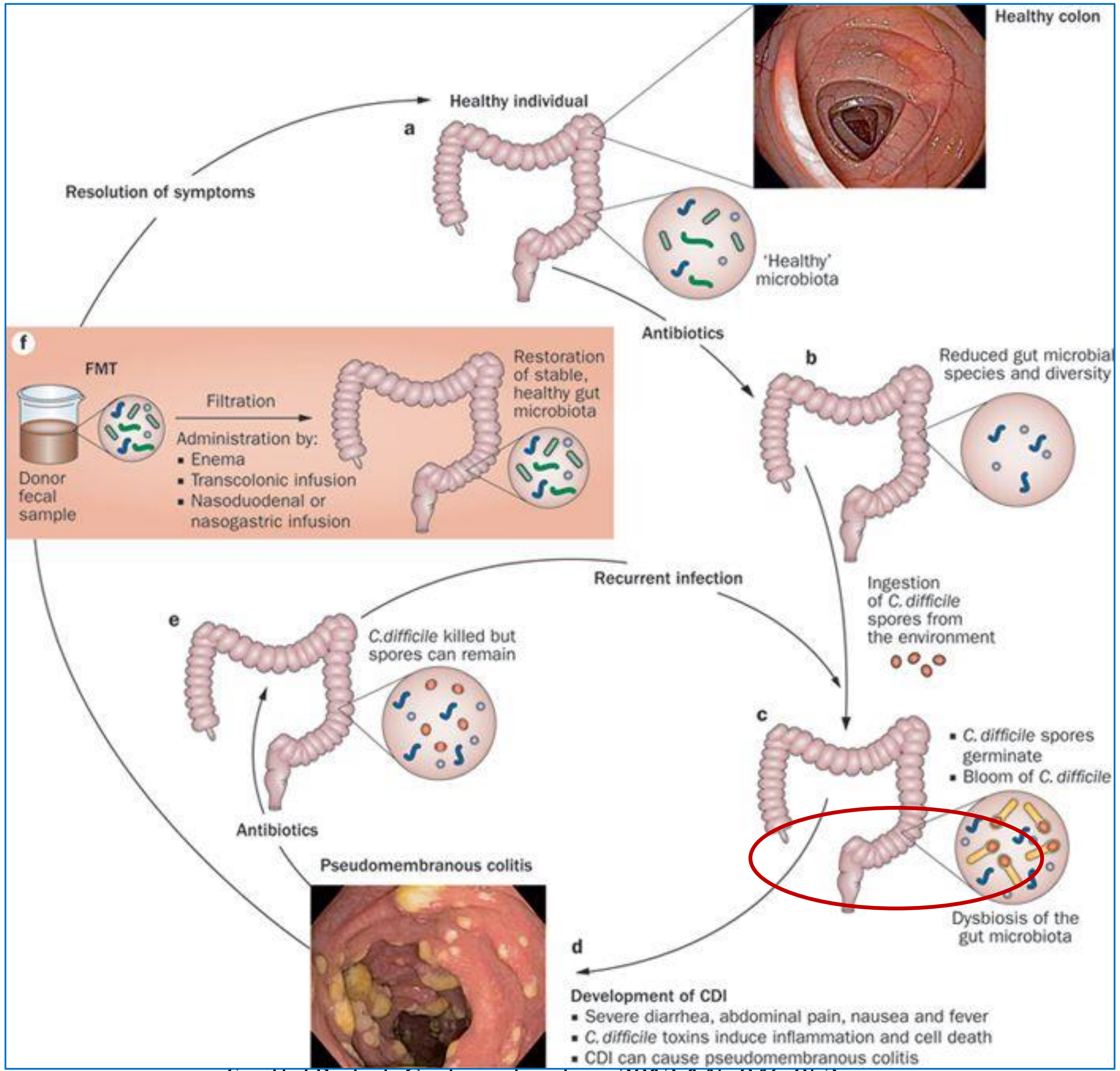
4. Fecal slurry is administered through colonoscopy

Routes of administration

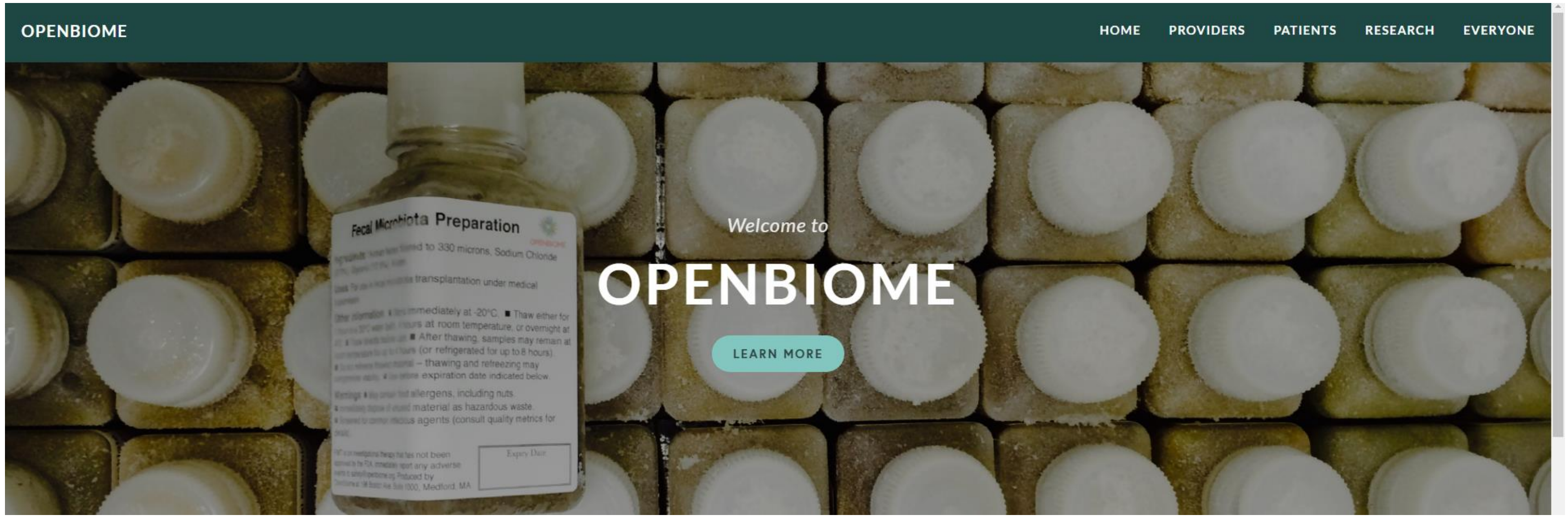


- nasogastric tube
- nasojejunal tube
- upper tract endoscopy (esophagogastroduodenoscopy)
- colonoscopy
- retention enema
- oral capsules
- **the best route most likely depends on the anatomic location of the disease**

Therapeutic potential of FMT



Smith LP et al. Gastroenterology 2013;145:946-953



- The Microbiome Health Research Institute, d.b.a. **OpenBiome, nonprofit organization dedicated to expanding safe access to fecal microbiota transplants (FMT), and to catalyzing research into the human microbiome.**

- Founded by a team of doctors, scientists and public health advocates, OpenBiome has two primary objectives:
    1. to eliminate the practical barriers to fecal microbiota transplantation
    2. to enable translational research into the human microbiome

https://www.openbiome.org/home/

Project APVV
TRANSMICROBIOM

Targeted modulation of gut microbiota and its transplantation in prevention and treatment of inflamantory bowel diseases

SLOVAK RESEARCH
AND DEVELOPMENT
AGENCY

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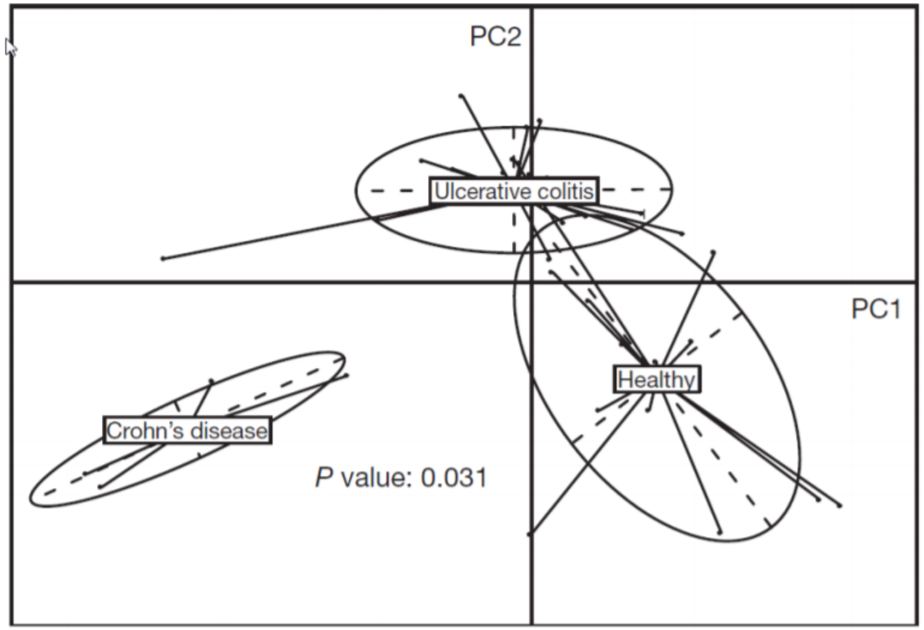
Bacterial species abundance differentiates IBD patients and healthy individuals



Microbial alterations

Decreased richness
Altered taxonomic profiles
Altered metabolic output

Qin *et al.* *Nature* **464**, 59-65 (2010)

Project partners

Institute of Experimental Medicine FM PJŠU in Košice

1st Departmnent of Internal Medicine, FM PJŠU and Louis Pasteur University Hospital in Košice

Institute of Biology and Ecology, Faculty of Science PJŠU

Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice

ProDigest, Belgium – technical & methodological support

TEKMAR Slovakia, Ltd.

Monsea, Ltd.

Goals & Objectives of project

1. Clarification of composition, diversity and functions of the healthy people‘ and IBD patients‘ gut microbiota

2. Study of the effect of faecal microbiota transplantation (healthy donor to recipient with IBD) on the composition and functions of the target gut microbiota using SHIME

3. Study the possibilities of targeted modulation of the microbiota in patients with IBD by its modification using SHIME and its reverse transfer

4. *In vivo* verification of the FMT and SHIME modulated IBD microbiota effectiveness in animal models (gnotobiotic mice associated with human microbiota and conventional rats)

THE PROJECT WORKFLOW

GUT MICROBIOME CHARACTERIZATION
Healthy & UC patients

Gut microbiome 16S metagenomics
examination, sampling, NGS, analysis

***IN VITRO* EXPERIMENTS**

GIT models, cell culture models, anaerobic microbiology
Gut microbiome simulation, *in vitro* modulation of UC dysbiosis, *in vitro* FMT testing

***IN VIVO* VERIFICATION**

Rat / mouse UC models
FMT method verification

1st phase

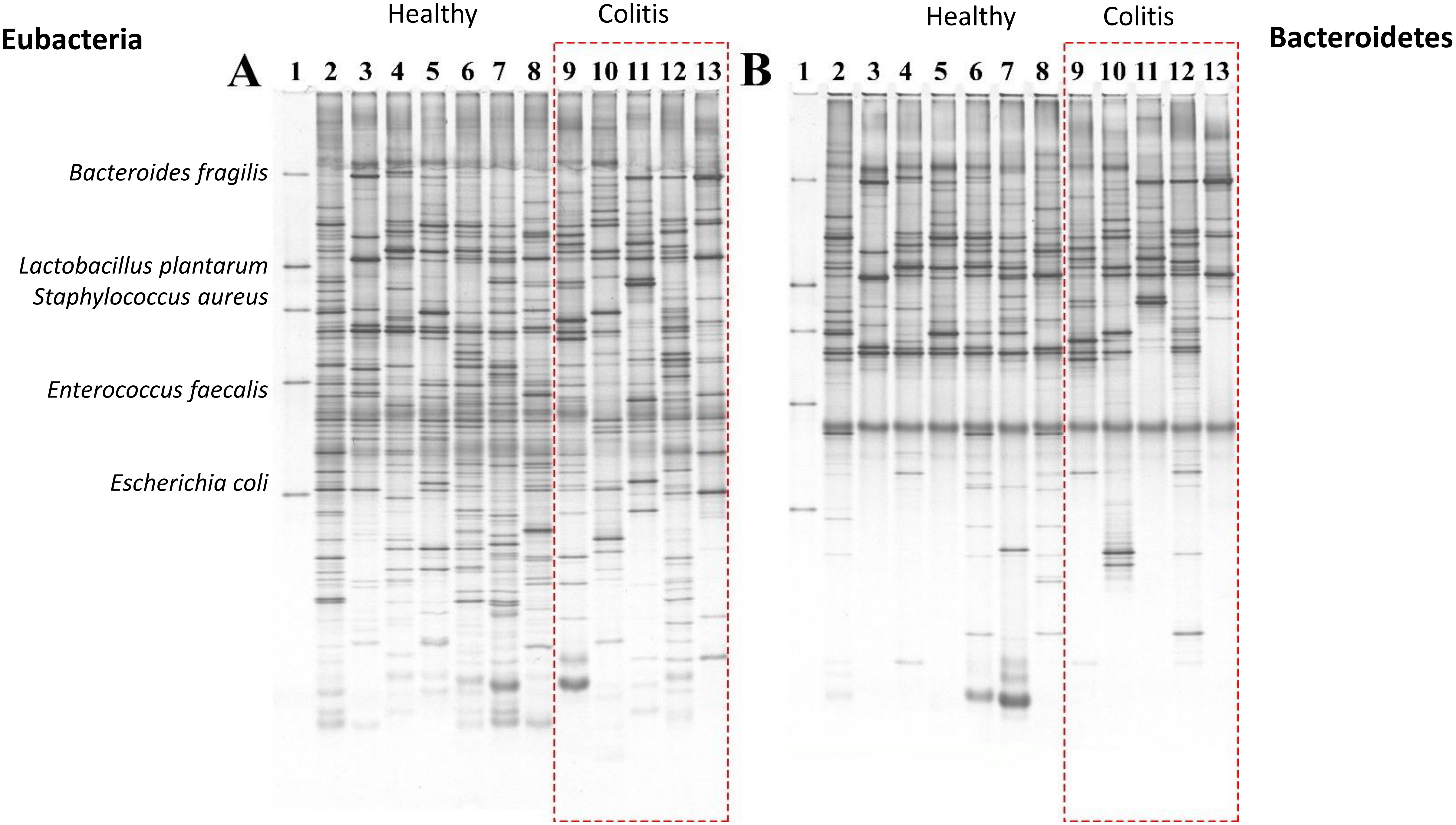
- Collection of samples (feces, blood)
- Study of the composition, function and diversity of the intestinal microbiota of healthy people and IBD patients
  - **Molecular, microbiological and biochemical analyses**
- Testing of various microbiota biomodulators (probiotic bacteria or natural bioactive substances) which could affect epithelial barrier integrity and immune functions in vitro
  - ***L. plantarum LS07, L. reuteri*, prebiotics, PUFA, etc.**

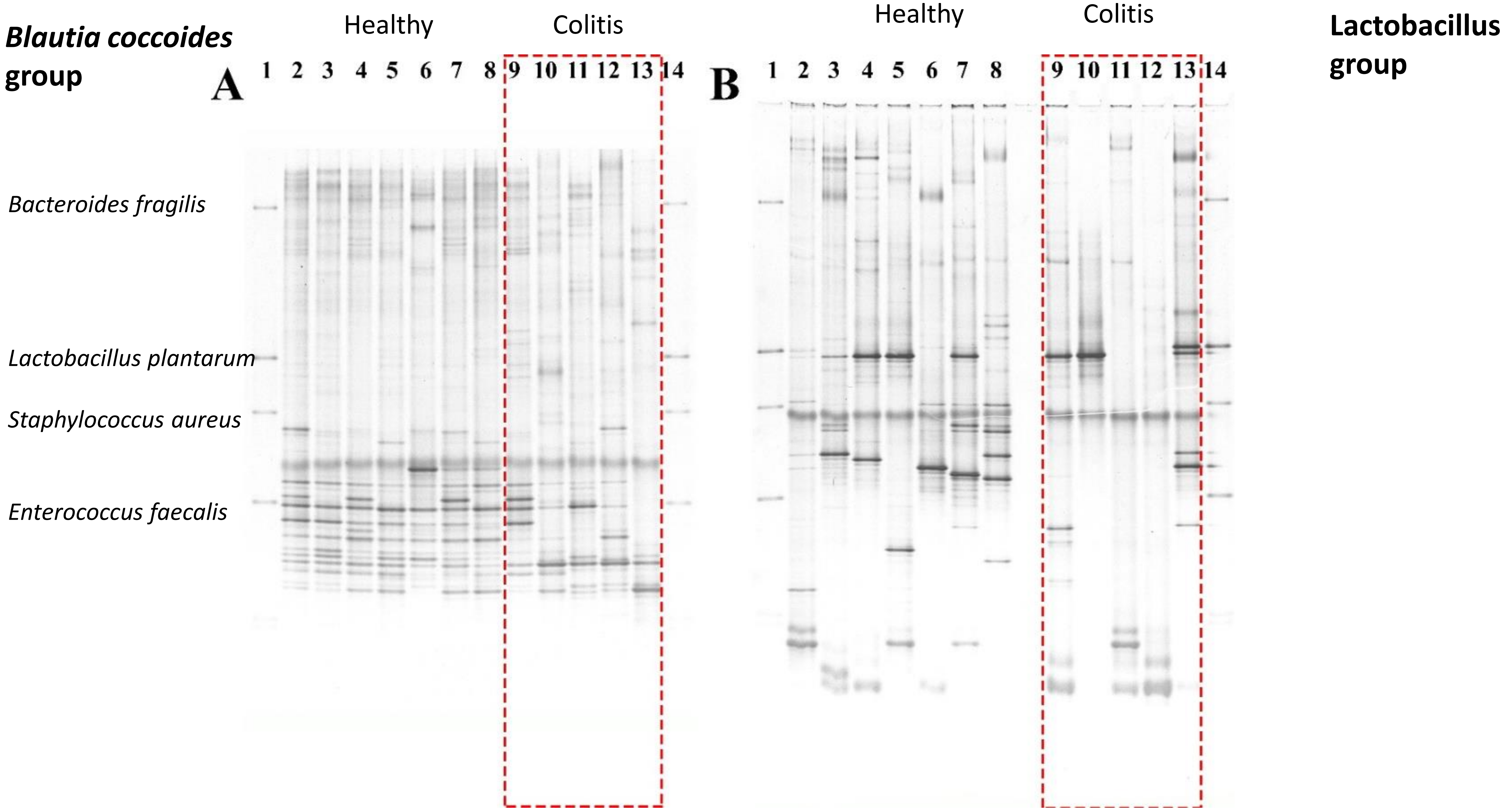
Microbial analyses of samples based on molecular methods

- isolation gDNA from stool samples
- qualitative characterization of microbiota composition (PCR-DGGE) *Euubacteria, Bacteroidetes, Lactobacillus and Clostridium(Blautia) coccoide* groups

- quantitative analyses of microbiome by real time qPCR
  - ➢ no significant diffrences in Eubacteria and Bacteroidetes

- NGS 16S rRNA sequencing (in progress)


Eubacteria
Healthy
Colitis
A
1 2 3 4 5 6 7 8 9 10 11 12 13
Bacteroides fragilis
Lactobacillus plantarum
Staphylococcus aureus
Enterococcus faecalis
Escherichia coli
Healthy
Colitis
Bacteroidetes
B
1 2 3 4 5 6 7 8 9 10 11 12 13


***Blautia coccoides* group**
Healthy
Colitis
A
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Bacteroides fragilis
Lactobacillus plantarum
Staphylococcus aureus
Enterococcus faecalis
Healthy
Colitis
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Lactobacillus group

Metabolic activity of gut microbiota

Microbial enzymatic analyses (fresh stool samples)

Spectrophotometric analyses of enzyme level:

β-glucuronidase

β -glucosidase

β-galactosidase

α-galactosidase

α-glucosidase

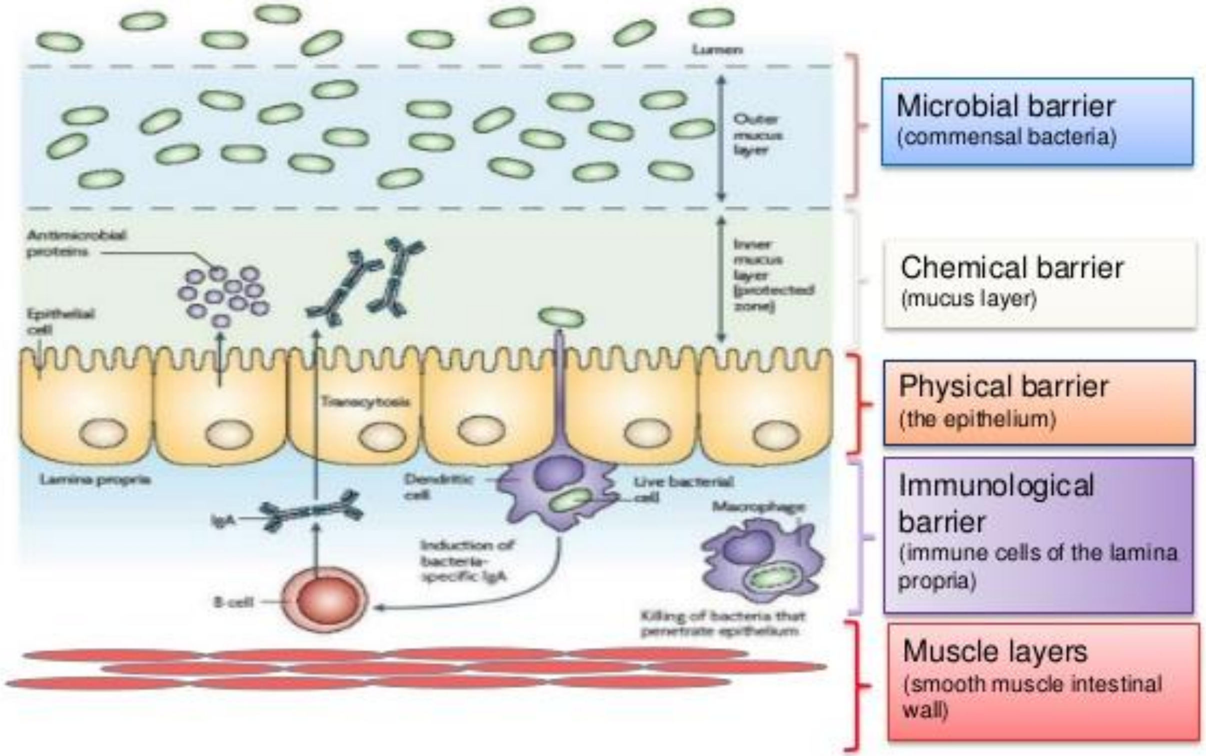
Biochemical analyses of organic acids in blood serum

- determination of short-chain fatty acids levels (acetic, propionic, butyric, valeric acid, and isovaleric acid, caproic acid and isocaproic) by **gas chromatography with flame ionization detector and mass-spectrophotometry**.

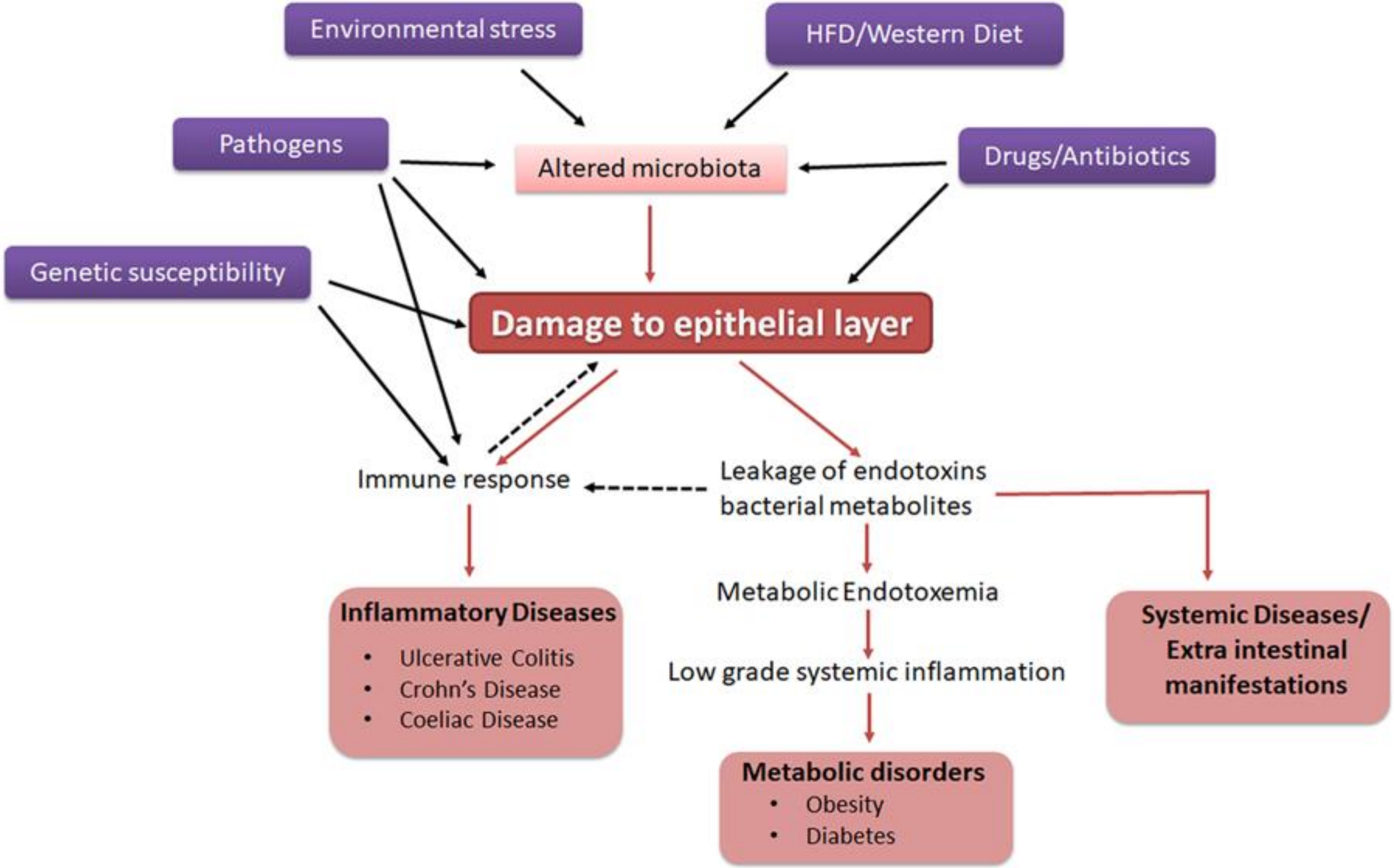
Testing of various microbiota biomodulators (probiotic bacteria or natural bioactive substances)

- which could affect epithelial barrier integrity and its function
- inhibit the pathogen adherence
- which could have immunomodulatory effect on M1 and M2 macrophages and PBMC

Gut barrier and microbiome



NATURE REVIEWS | **MICROBIOLOGY VOLUME 14 | JANUARY 2016 | 21**



Environmental stress

HFD/Western Diet

Pathogens

Altered microbiota

Drugs/Antibiotics

Genetic susceptibility

Damage to epithelial layer

Immune response

Leakage of endotoxins bacterial metabolites

Metabolic Endotoxemia

Low grade systemic inflammation

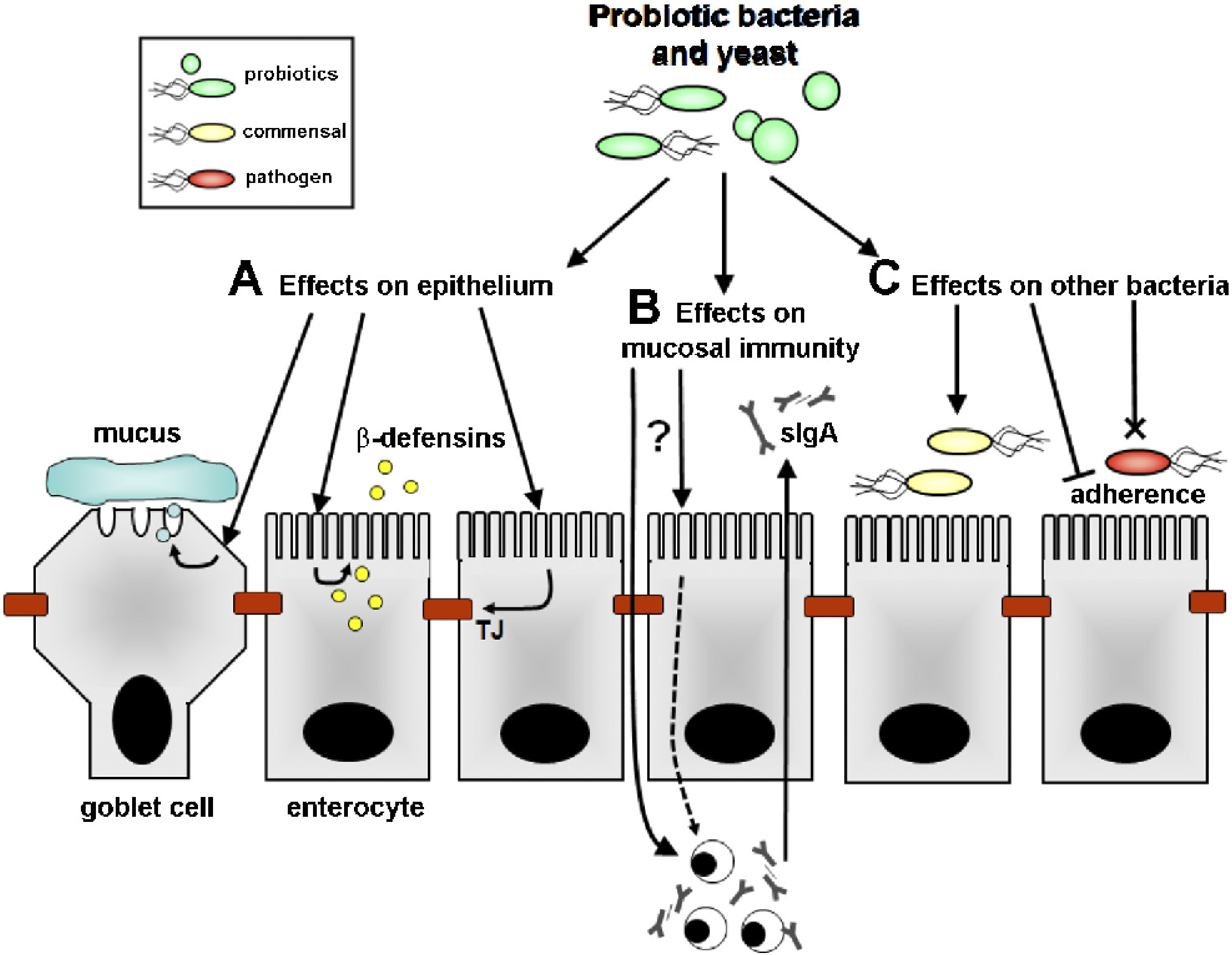
Inflammatory Diseases

- Ulcerative Colitis
- Crohn’s Disease
- Coeliac Disease

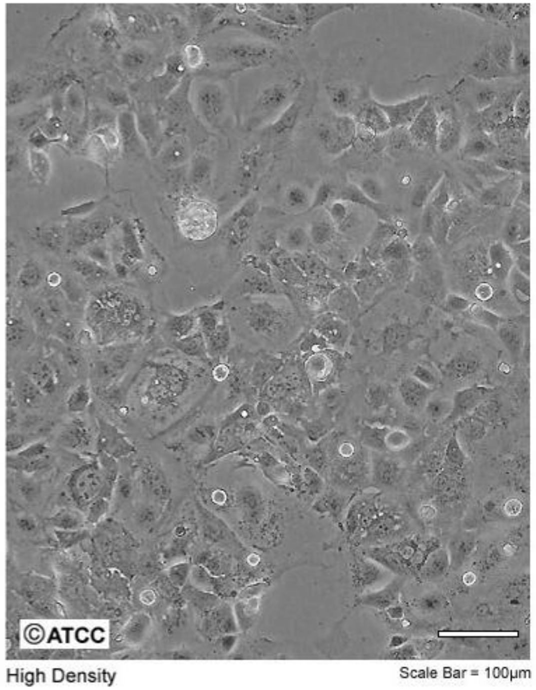
Systemic Diseases/ Extra intestinal manifestations

Metabolic disorders

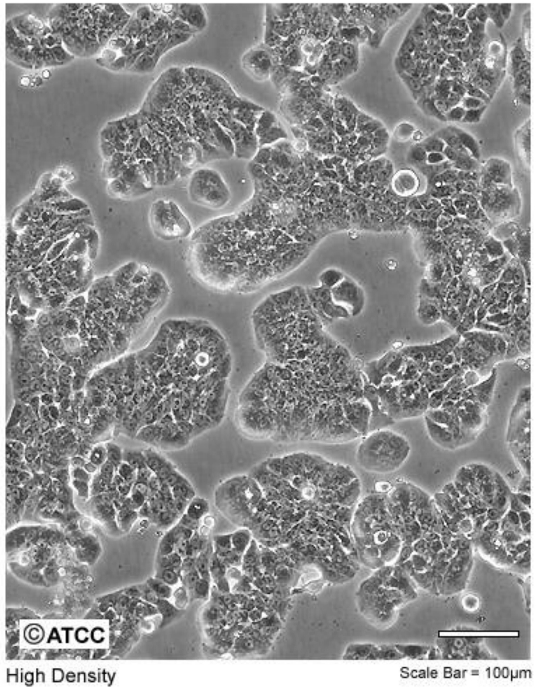
- Obesity
- Diabetes


probiotics
commensal
pathogen
Probiotic bacteria and yeast
A Effects on epithelium
B Effects on mucosal immunity
C Effects on other bacteria
mucus
β-defensins
?
sIgA
adherence
TJ
goblet cell
enterocyte

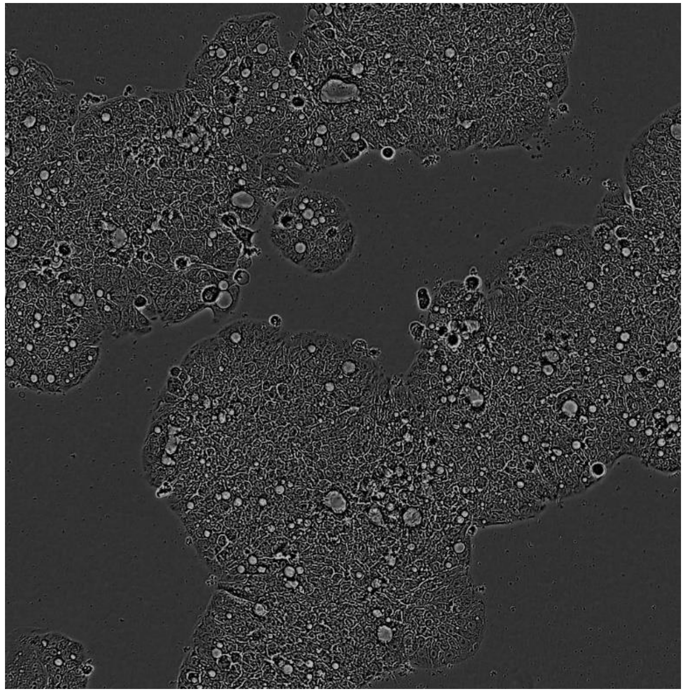
Caco-2



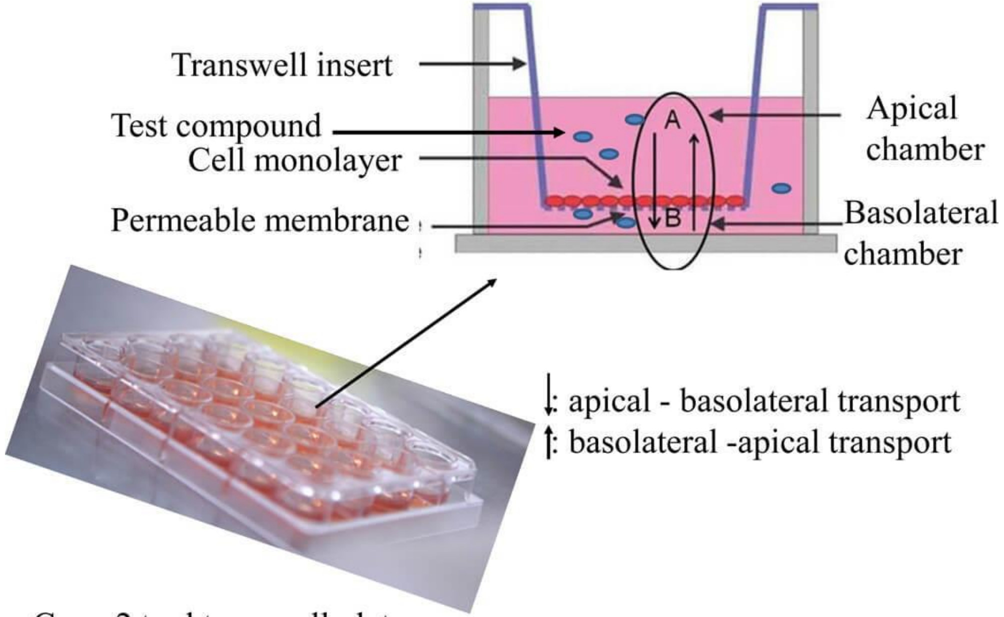
HT-29



HT-29-MTX

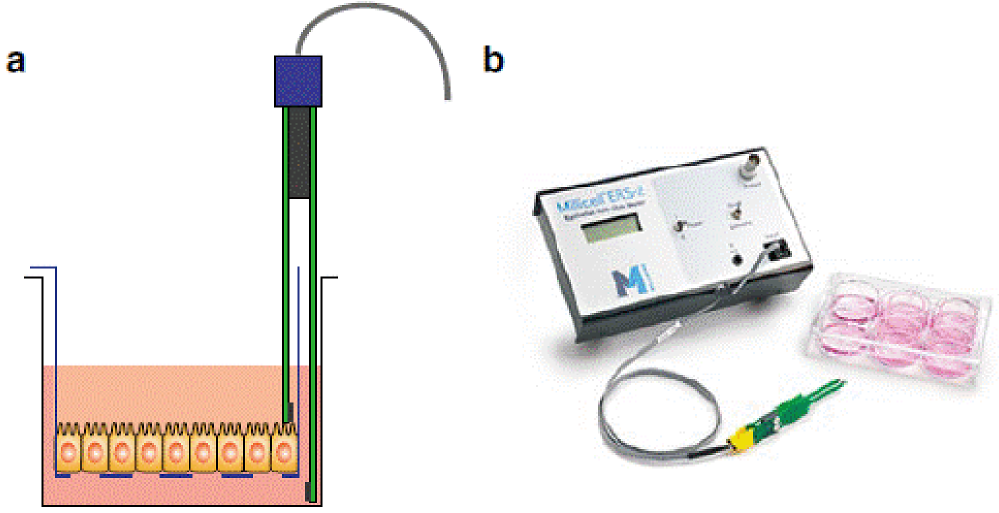


In vitro models of gut barier based on the immortalised epithelial cell lines cultivation

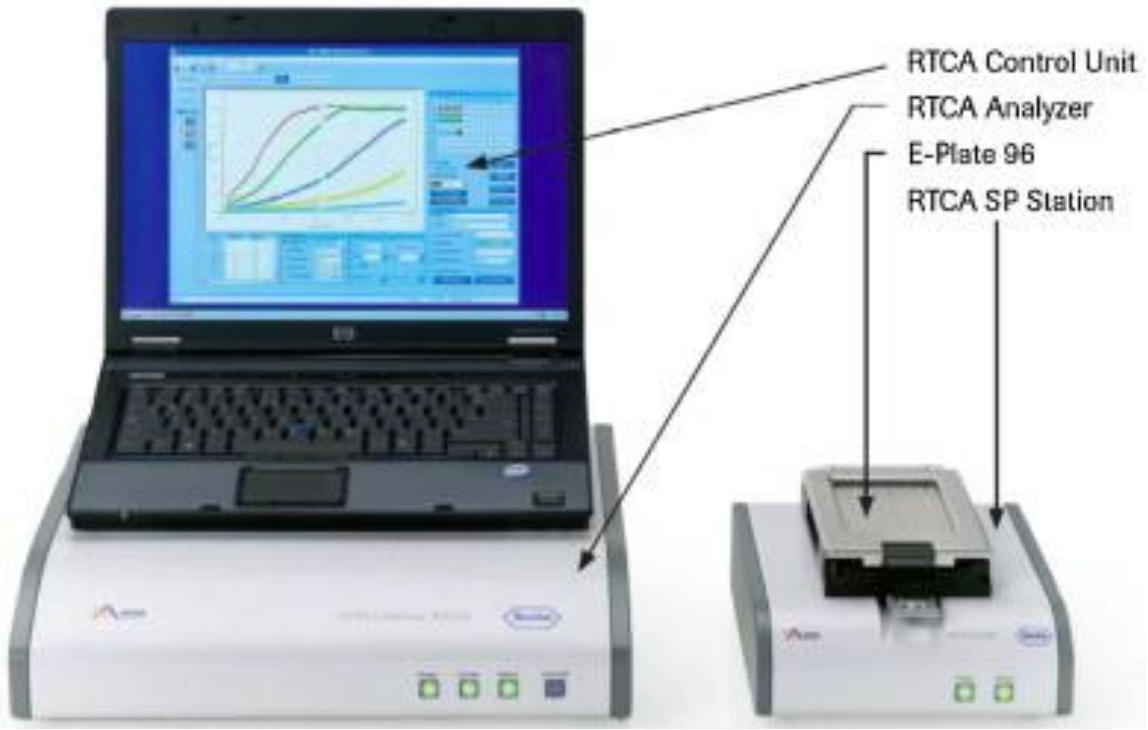


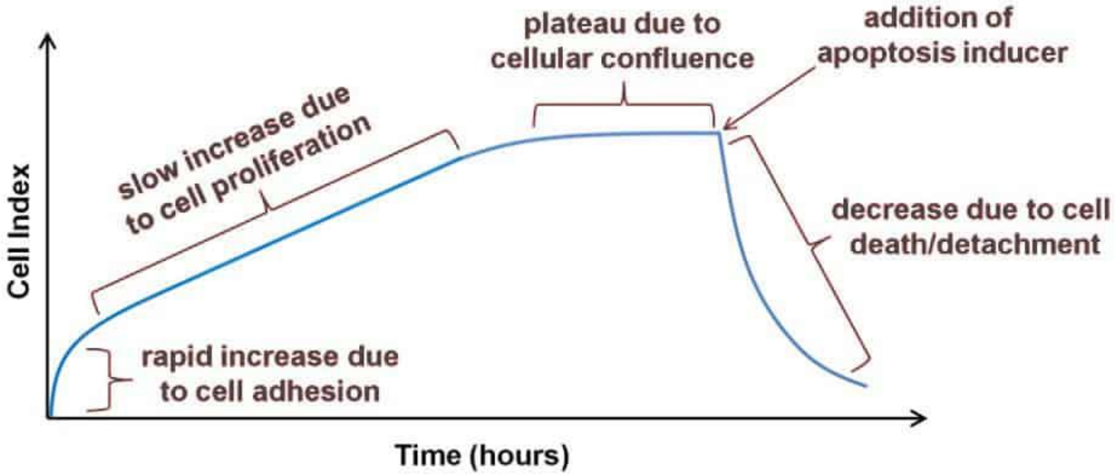
Caco-2 tool transwell plate

Measurement of transepithelial electrical resistance (TEER) of cells growing on a microporous membrane

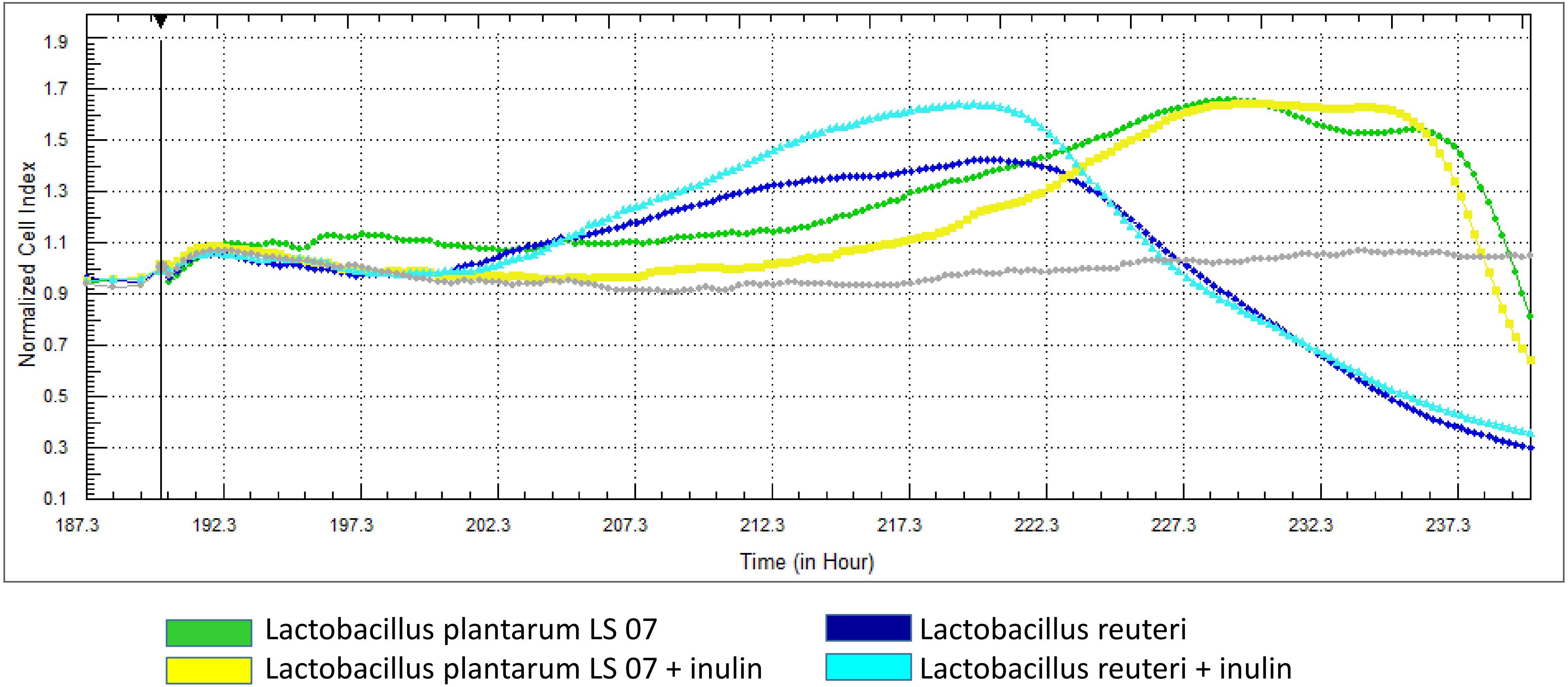


xCELLigence SP RTCA system for real time monitoring the intestinal barrier function



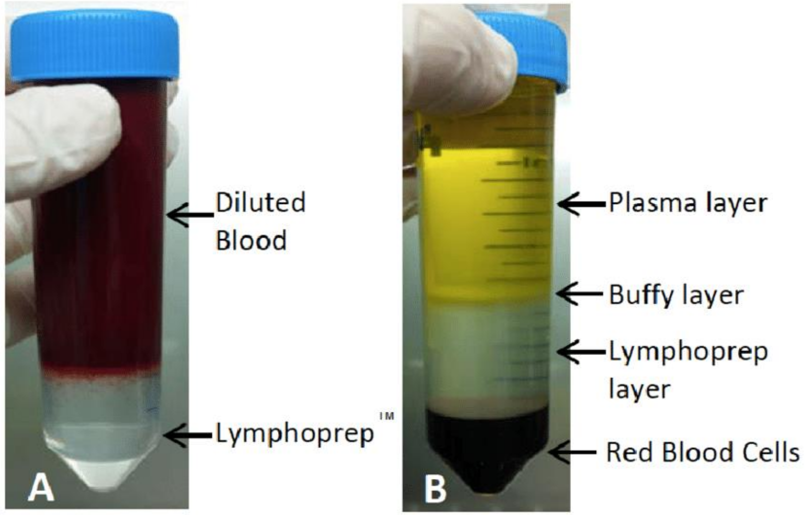


Effect of lactobacili and inulin on gut barrier



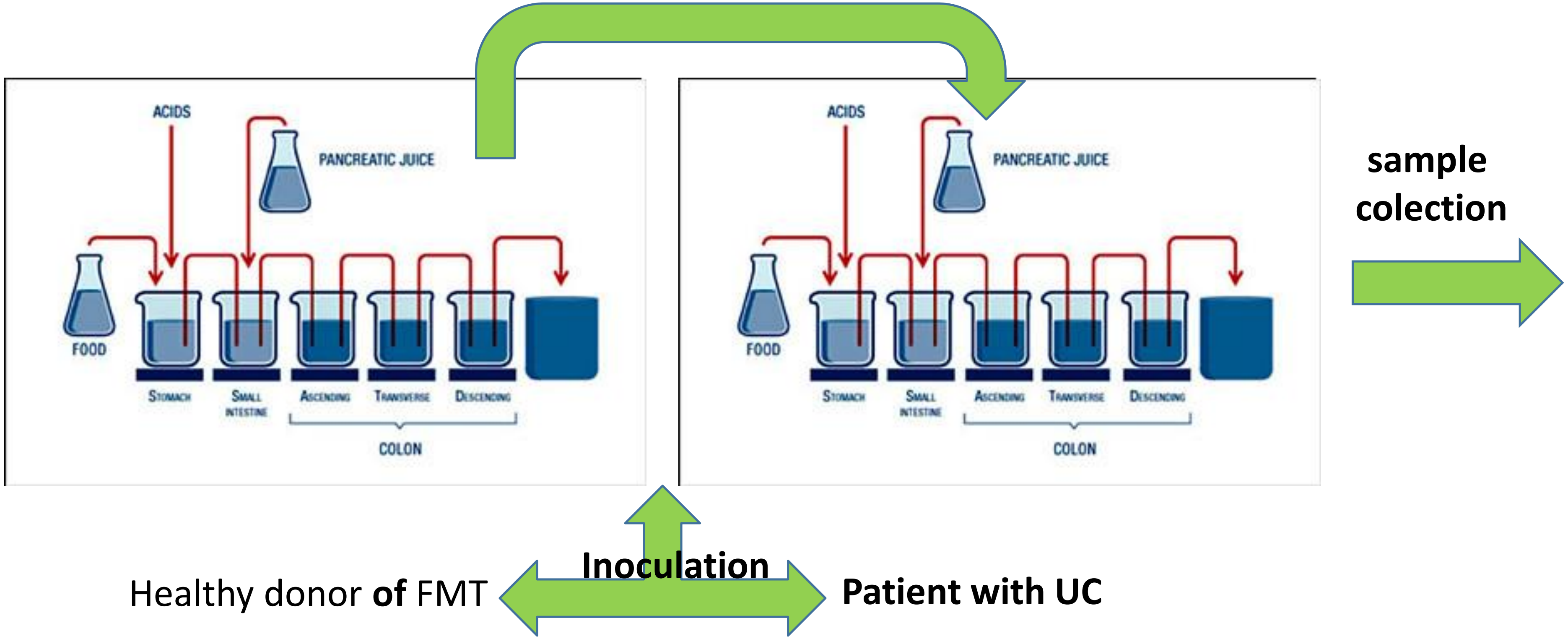
Imunomodulatory effect of probiotics and natural substances

- **THP-1 monocytic line** – differentiation on M1 or M2 macrophages, phagocytic activity, cytokine production after 24h bacterial stimulation
- **Peripheral blood mononuclear cells** isolated from healthy human and patients with UC – cytokine production after stimulation with bacteria



2nd phase:

- **Study of the effect of healthy donors FMT on the IBD patients‘ microbiota composition and functionality using *in vitro* TWINSHIME**



Microbiota Transfer

sample colection

Inoculation

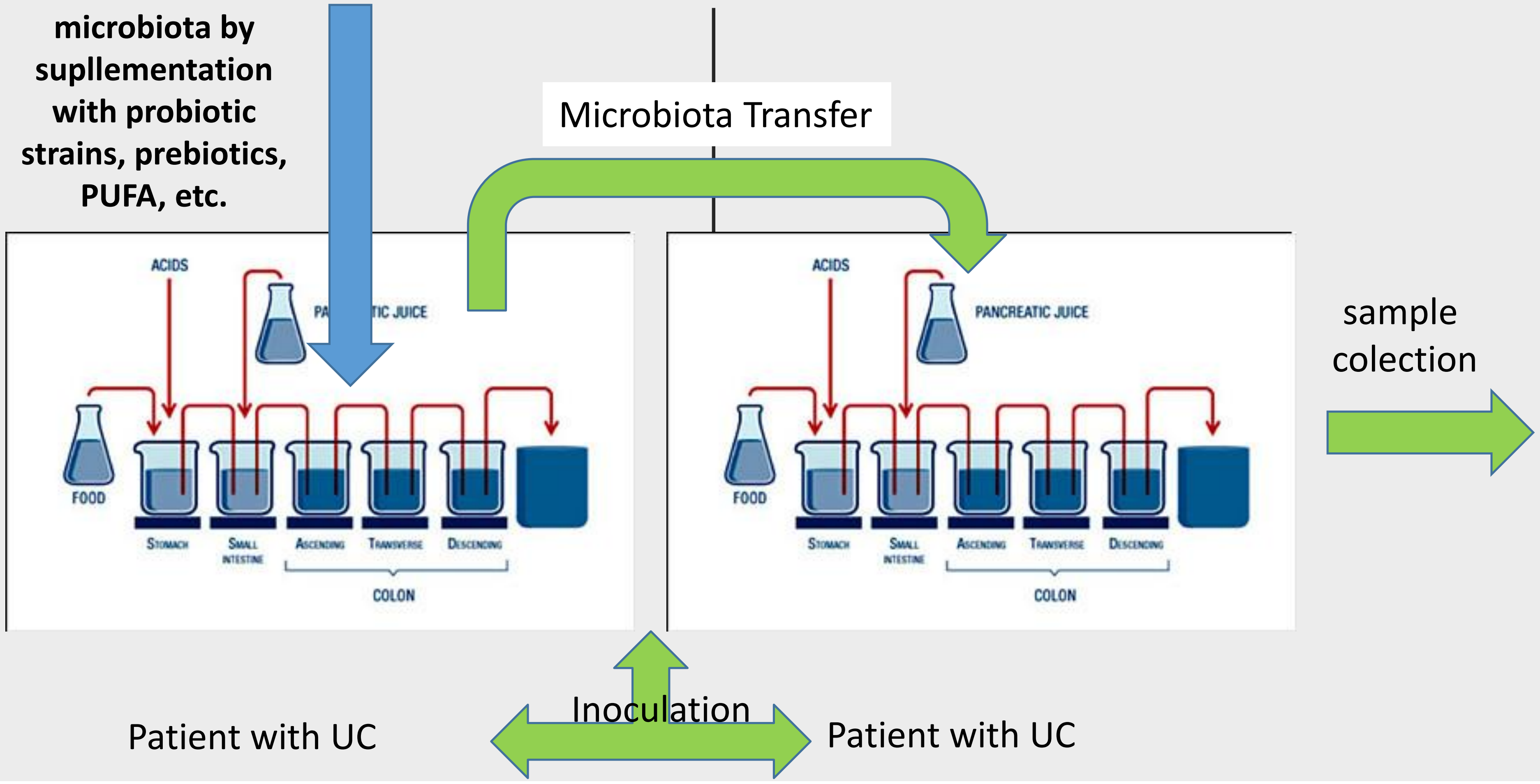
Healthy donor **of** FMT

Patient with UC

Molecular analysis of gut microbiota composition (DGGE, qPCR)
Biochemical and metabolic analysis of microbiota activity

3rd phase:

- Detection of optimal biomodulators for targeted modulation of IBD microbiota using *in vitro* TWINSHIME
- **Return transfer of the IBD patients' modulated microbiota to native IBD microbiota using *in vitro* TWINSHIME**



Modulation of microbiota by supllementation with probiotic strains, prebiotics, PUFA, etc.

Microbiota Transfer

sample colection

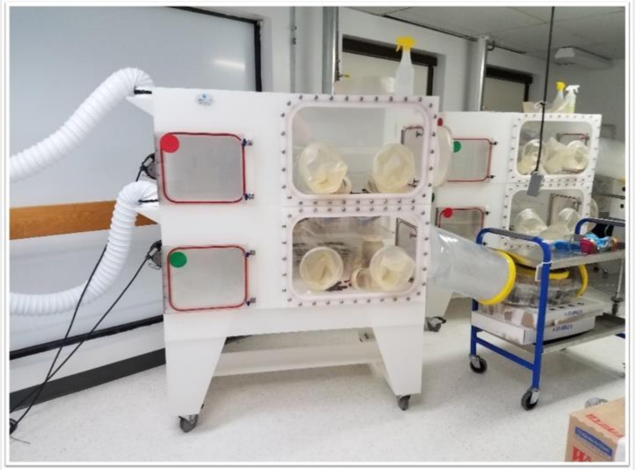
Molecular analysis of gut microbiota composition (DGGE, qPCR)
Biochemical and metabolic analysis of microbiota activity

Inoculation

Patient with UC

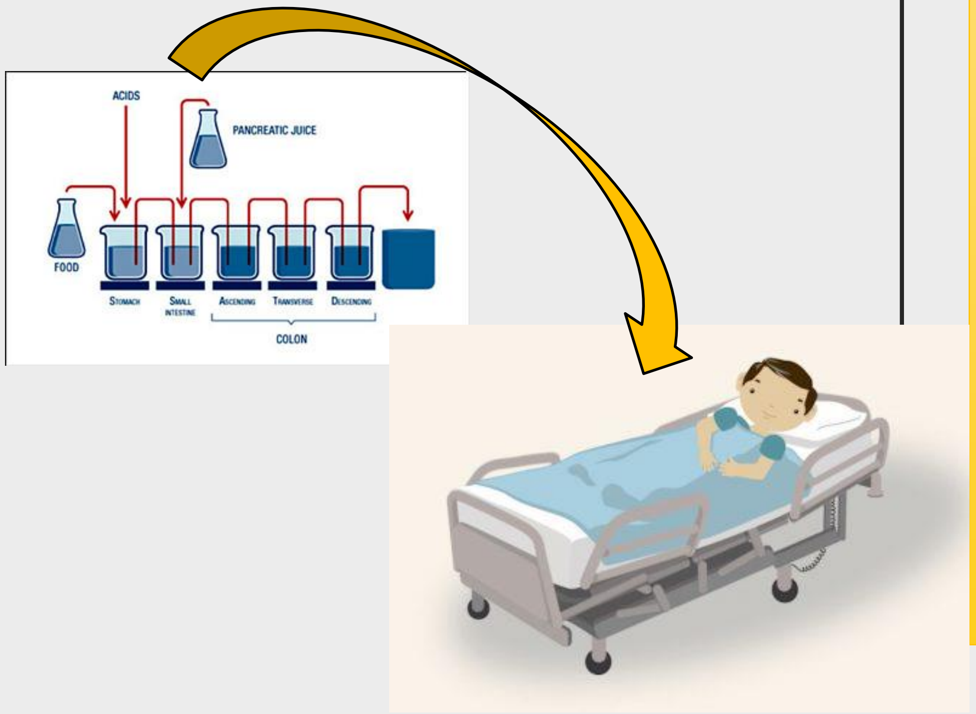
Patient with UC

4th phase:



- ***In vivo* verification of the FMT and SHIME modulated IBD microbiota effectiveness in animal models (gnotobiotic mice associated with human microbiota and conventional rats)**
  - UC chemicaly-induced by DSS
  - analyses of changes in gut microenvironment (microbiological, biochemical, physiological parameters of the metabolism and utilization of nutrients) & morfological and immunological parameters

EXPECTED RESULTS



- Original solution of gut microbiota modulation which could possibly meet criteria of the **personalized medicine approach**

- Our solution **eliminates the risks** connected with the fecal microbiota transplantation from donor and allows **targeted modulation according to specific needs of the patient**

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Thank you for your attention.