Evaluation of probiotics and prebiotics in food

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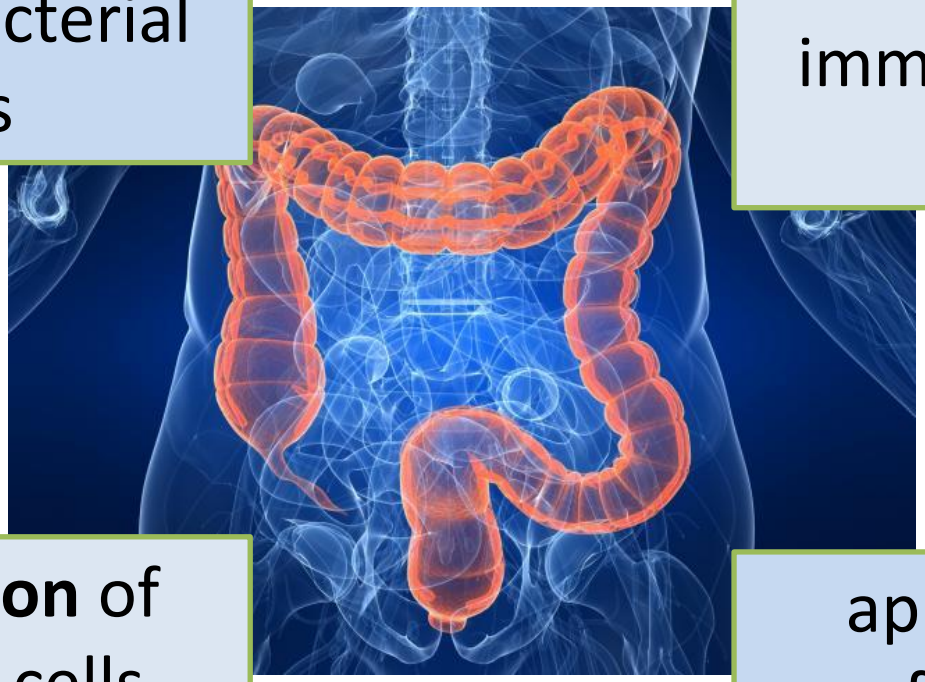
MICROBIOTA - ‘MICROFLORA OF THE BODY’

- ✓ Humans have got approximately **10^{13} cells of their body**.
- ✓ Humans have got around **10^{14} bacterial cells in/on their body**.
- ✓ Members of groups such as **viruses, fungi and protozoa** are also regularly found in healthy individuals, but form only a minor component of the total population of resident organisms.
- ✓ The microorganisms occur in parts of the body that are exposed to / or communicate with the external environment (the skin, nose and mouth, and intestinal and uriogenital tracts etc.).
- ✓ **The majority of bacterial cells is associated with the gastrointestinal tract.**
- ✓ **Internal organs and tissues are normally sterile.**

Intestinal mikrobiota – forgotten organ

10^{14} of bacterial cells

60 – 70 % of immune system cells



100 million of nervous cells

approximate surface **300 m²**

BENEFITS OF MICROBIOTA FOR THEIR HOST

Structural functions:

Barrier fortification

Induction of IgA

Apical tightening of tight junctions

Immune system development

Protective functions:

Pathogen displacement

Nutrient competition

Receptor competition

Production of anti-microbial factors

e.g., bacteriocins, lactic acids

Metabolic functions:

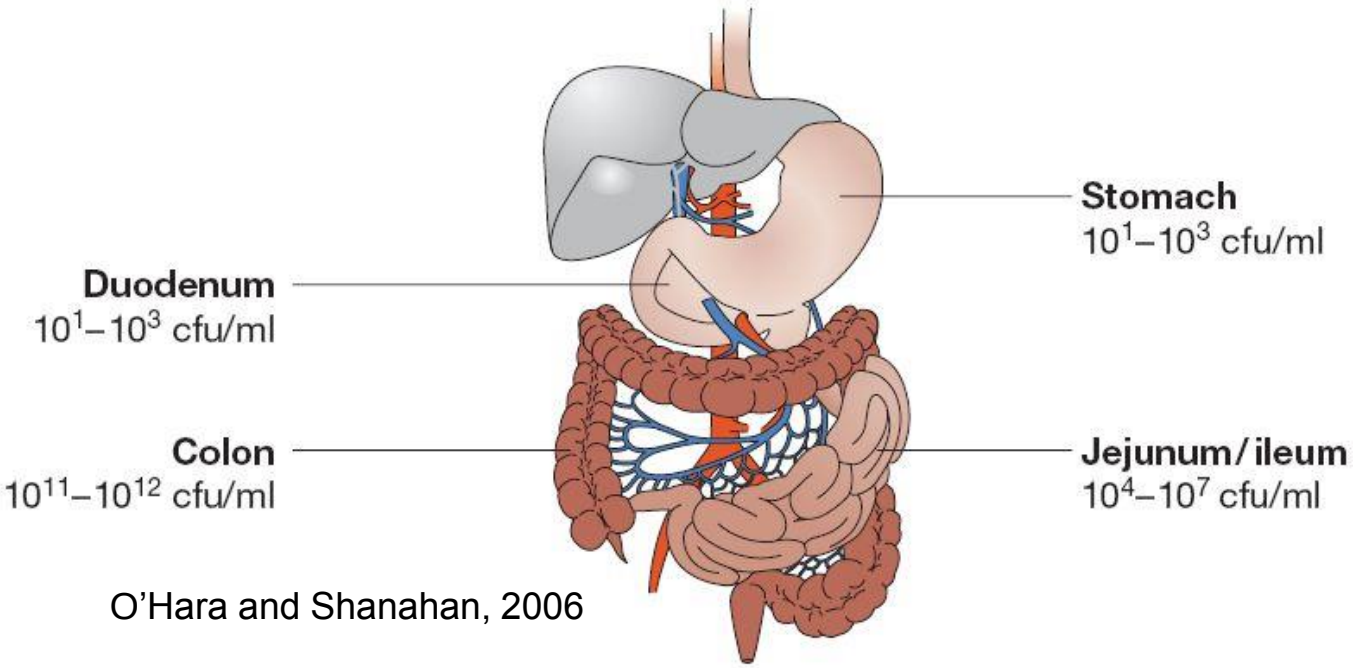
Control intra epithelial cell differentiation and proliferation

Metabolize dietary carcinogens

Synthesize vitamins e.g., biotin, folate

Ferment non-digestible dietary residue and endogenous epithelial derived mucus Ion absorption

BACTERIAL COLONIZATION OF THE GASTROINTESTINAL TRACT



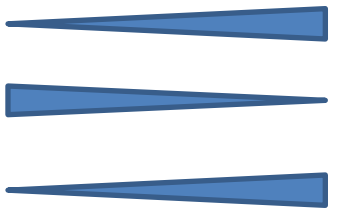
O'Hara and Shanahan, 2006

Anaerobic genera
Bifidobacterium
Clostridium
Bacteroides
Eubacterium

Aerobic genera
Escherichia
Enterococcus
Streptococcus
Klebsiellac

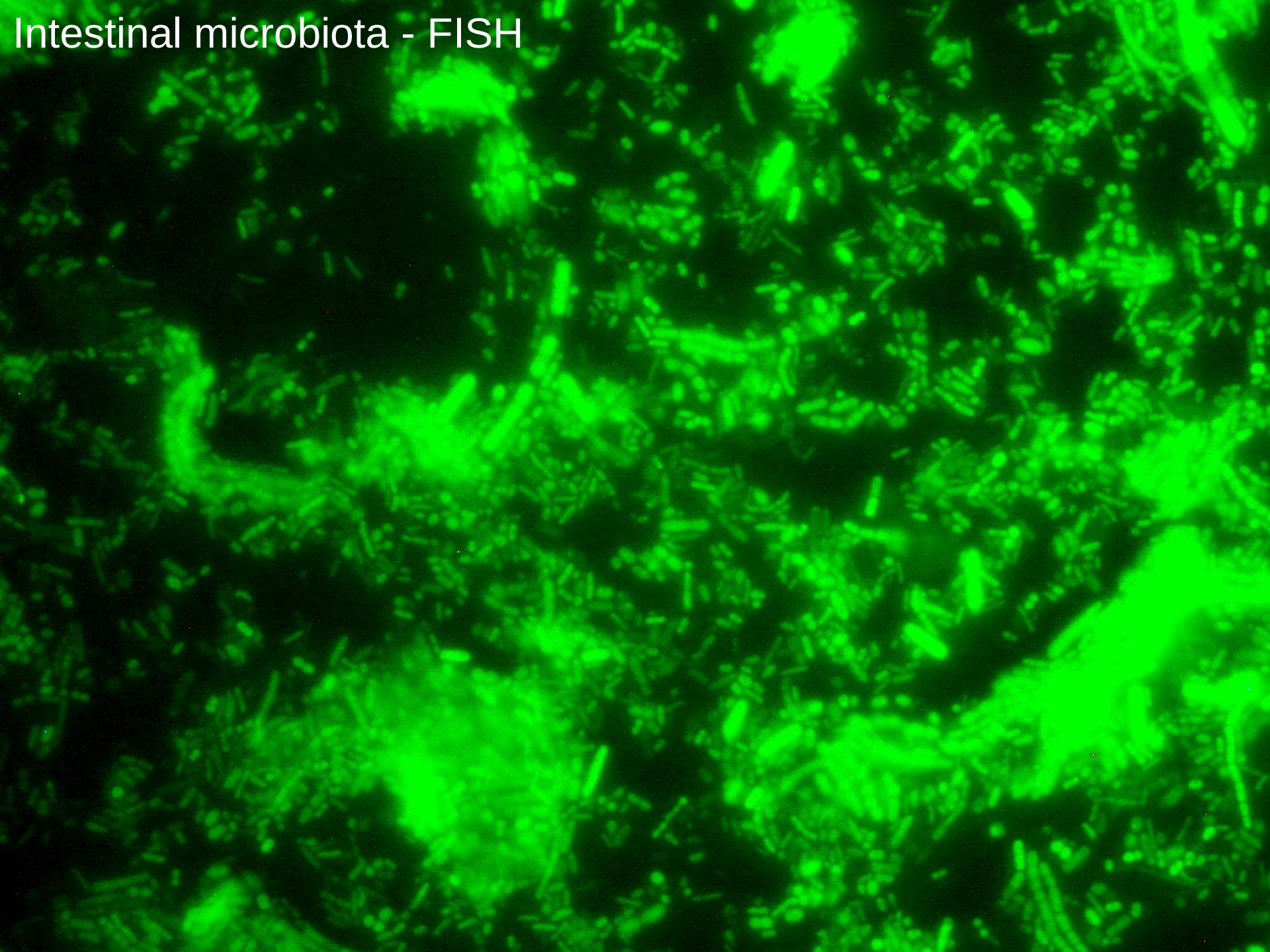
MOUTH → ***ANUS***

- ✓ Number of bacterial CFU is caudally increasing
- ✓Number of **aerobic** bacteria is caudally decreasing
- ✓ Number of **anaerobic** bacteria is caudally increasing

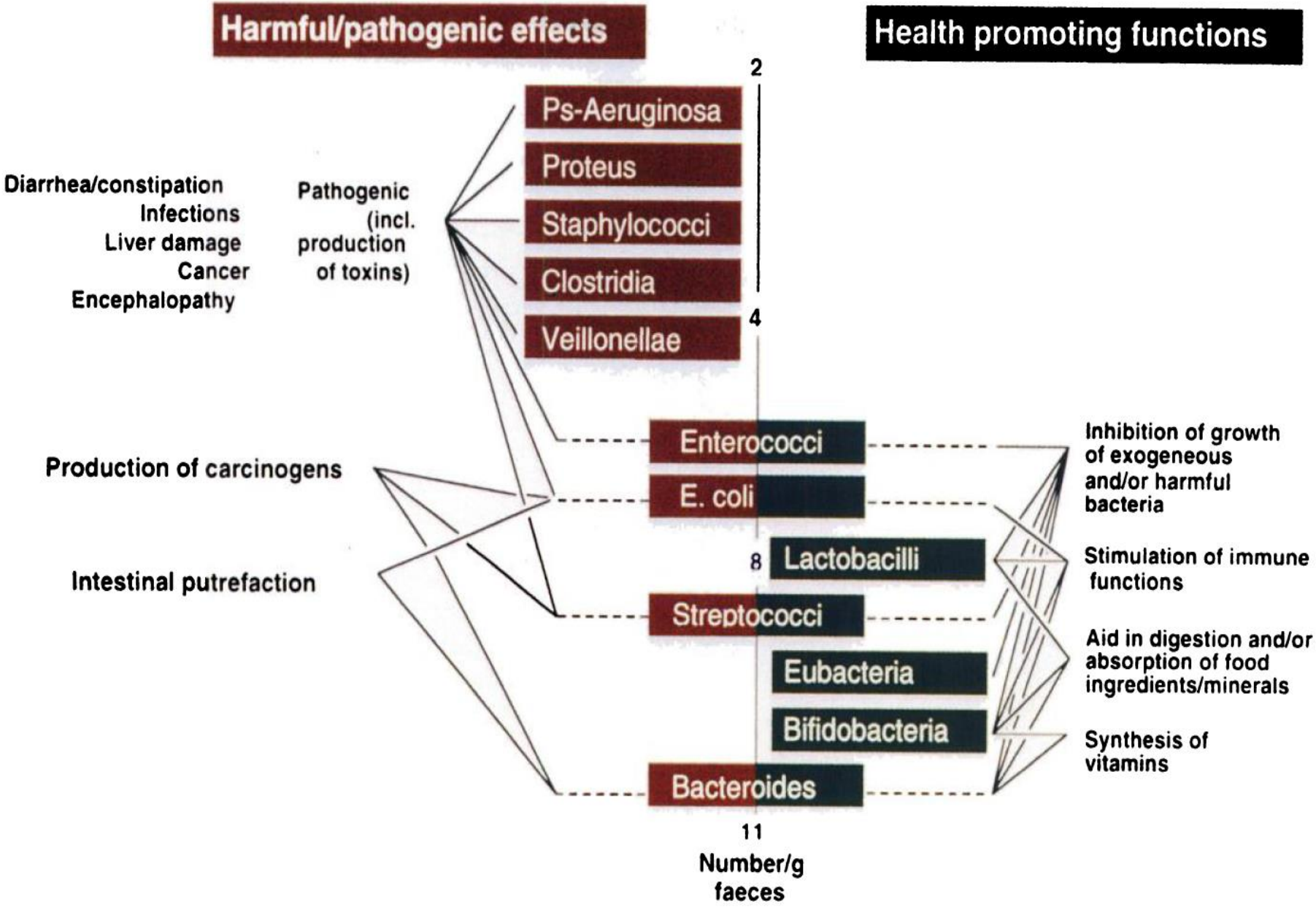


CFU = Colony Forming Units

Intestinal microbiota - FISH



Health effect of predominant intestinal bacteria



The gut microbiota through different life-stages

S.H. Duncan, H.J. Flint / Maturitas 75 (2013) 44–50

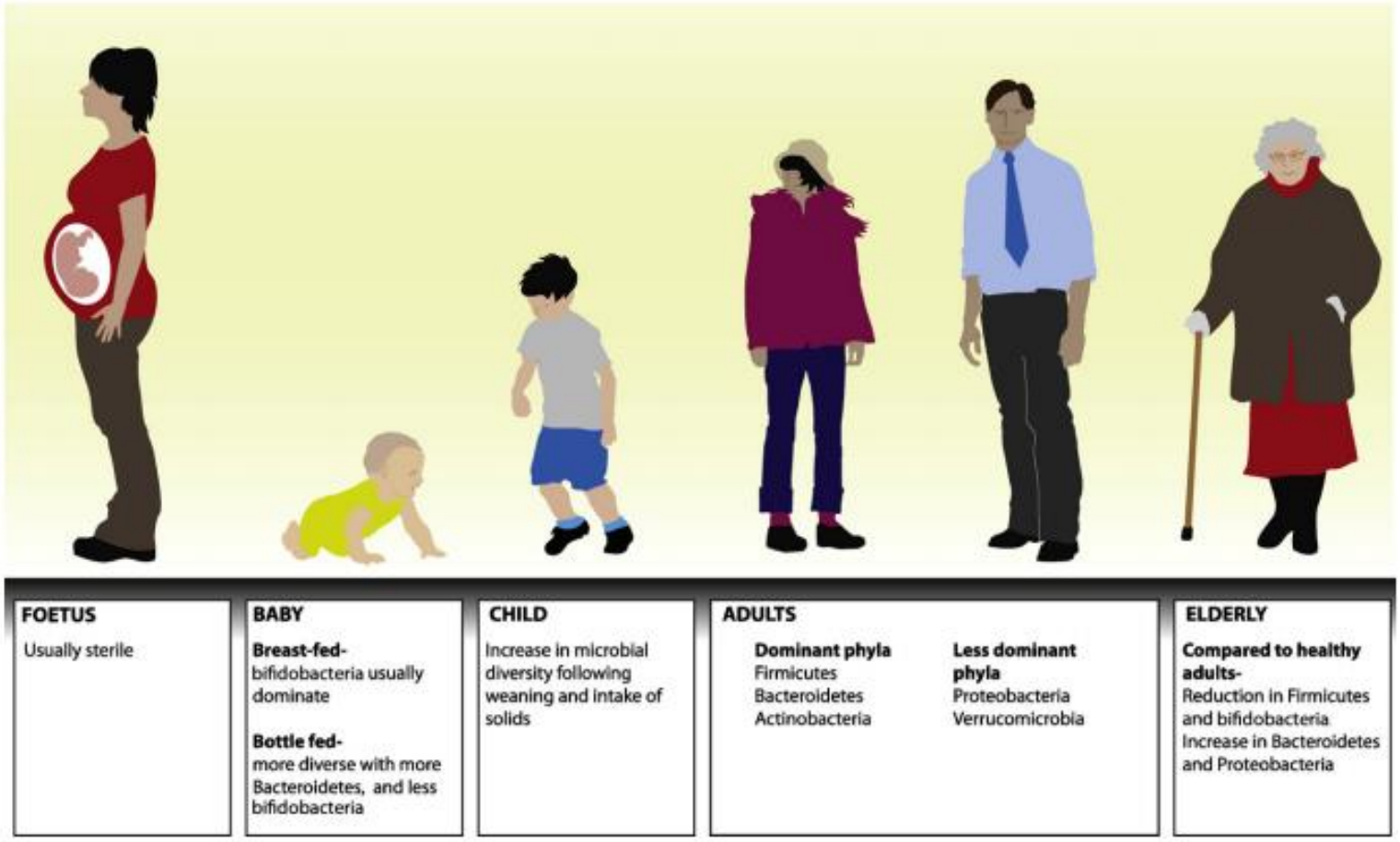
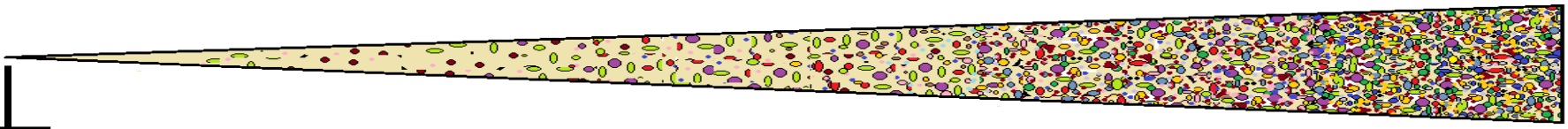


Fig. 1. Schematic summarising changes in the composition of the gut microbiota through different life-stages.

The colonization of the intestine



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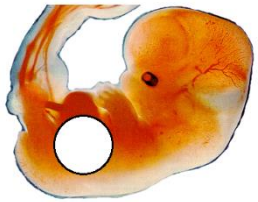
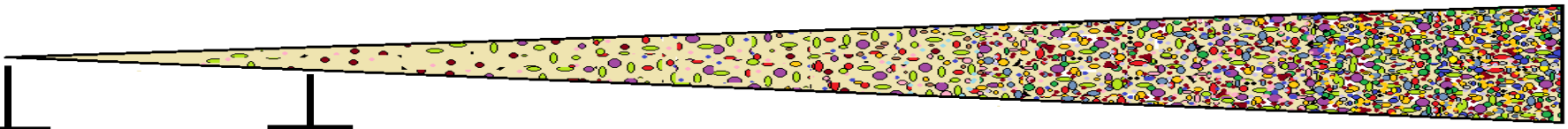


Sterile gut?

Specific microbiota in the placenta

FETUS
placental microbiome

The colonization of the intestine



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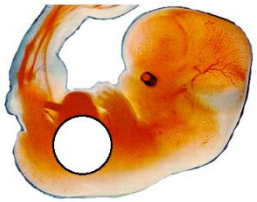

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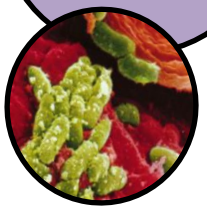
placental microbiome

BIRTH
1. Contact with vaginal-, intestinal-, skin-flora

Caesarean section

Skin flora

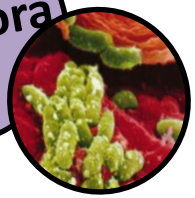


Spontaneous delivery (vaginal)

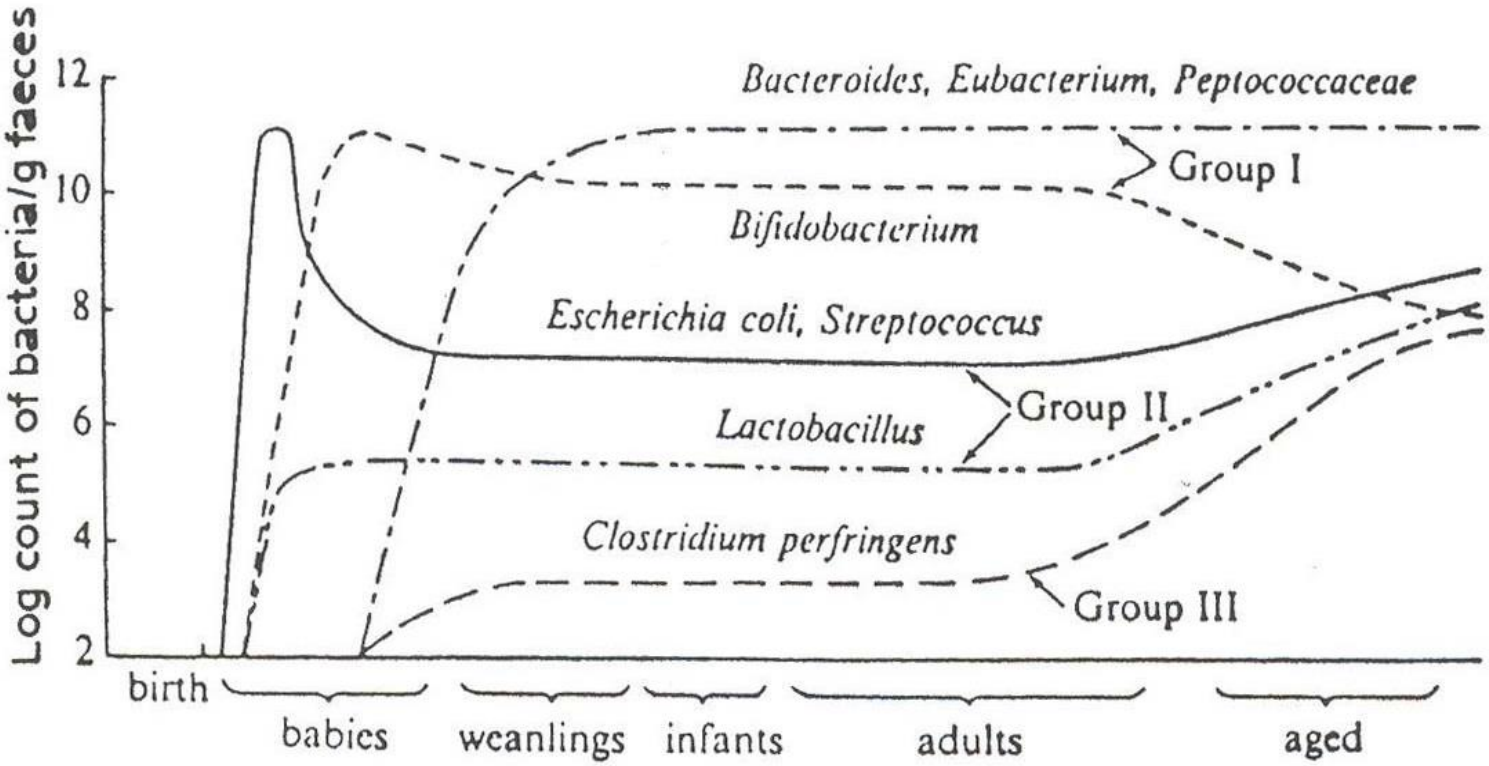
Vaginal flora

Intestinal flora

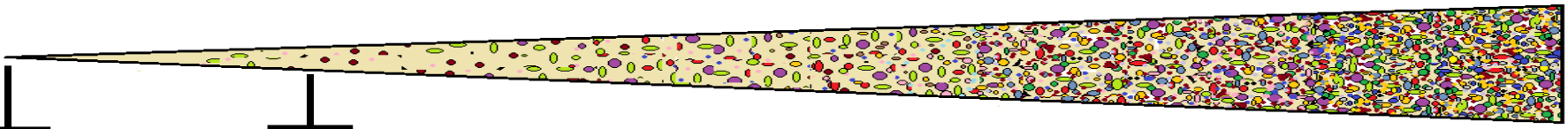
Skin flora



Changes in faecal flora during life (Mitsuoka, 1992)



The colonization of the intestine



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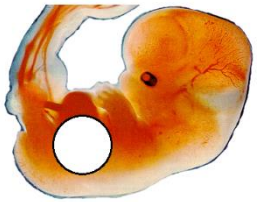

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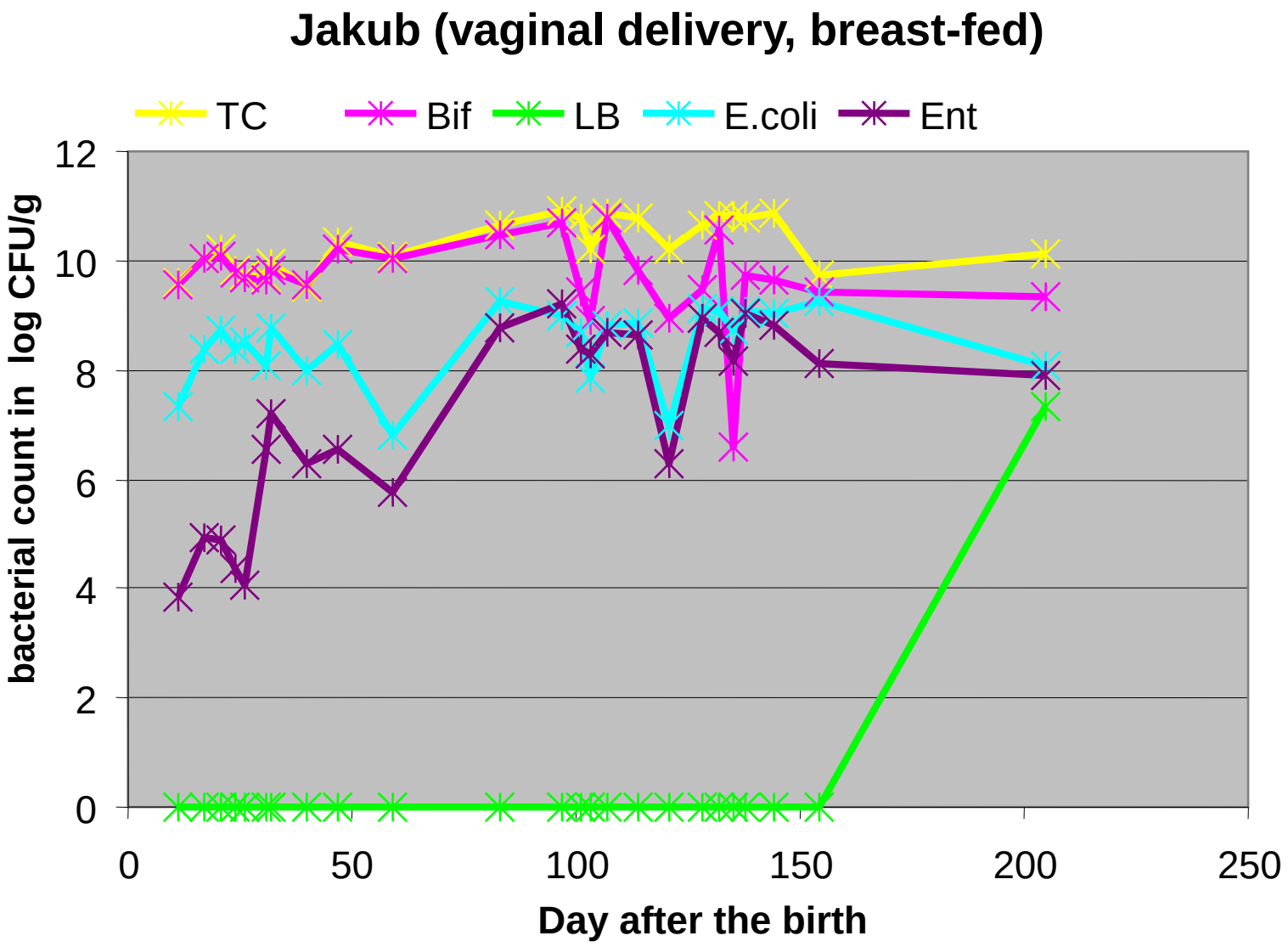
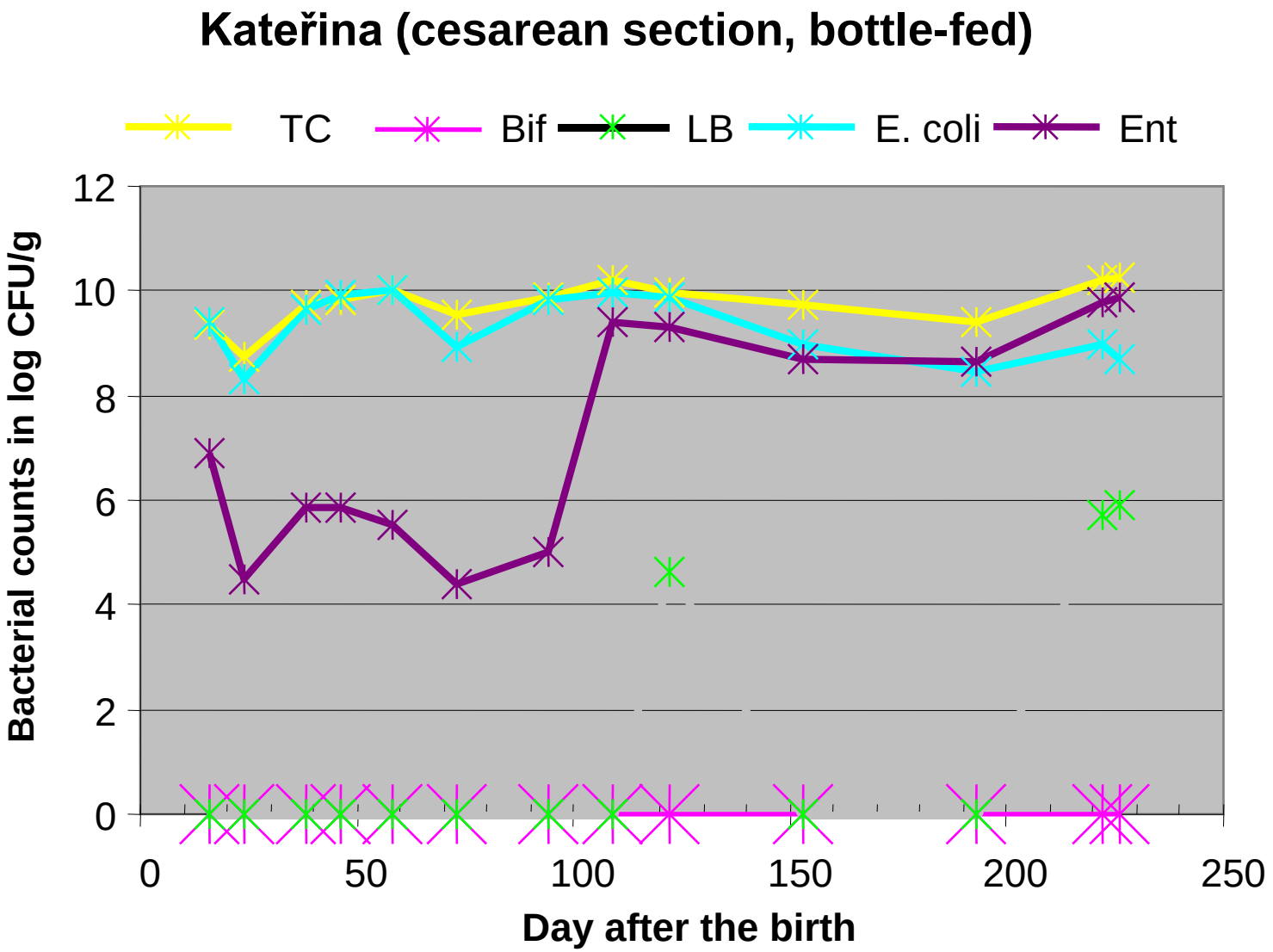
Breast milk

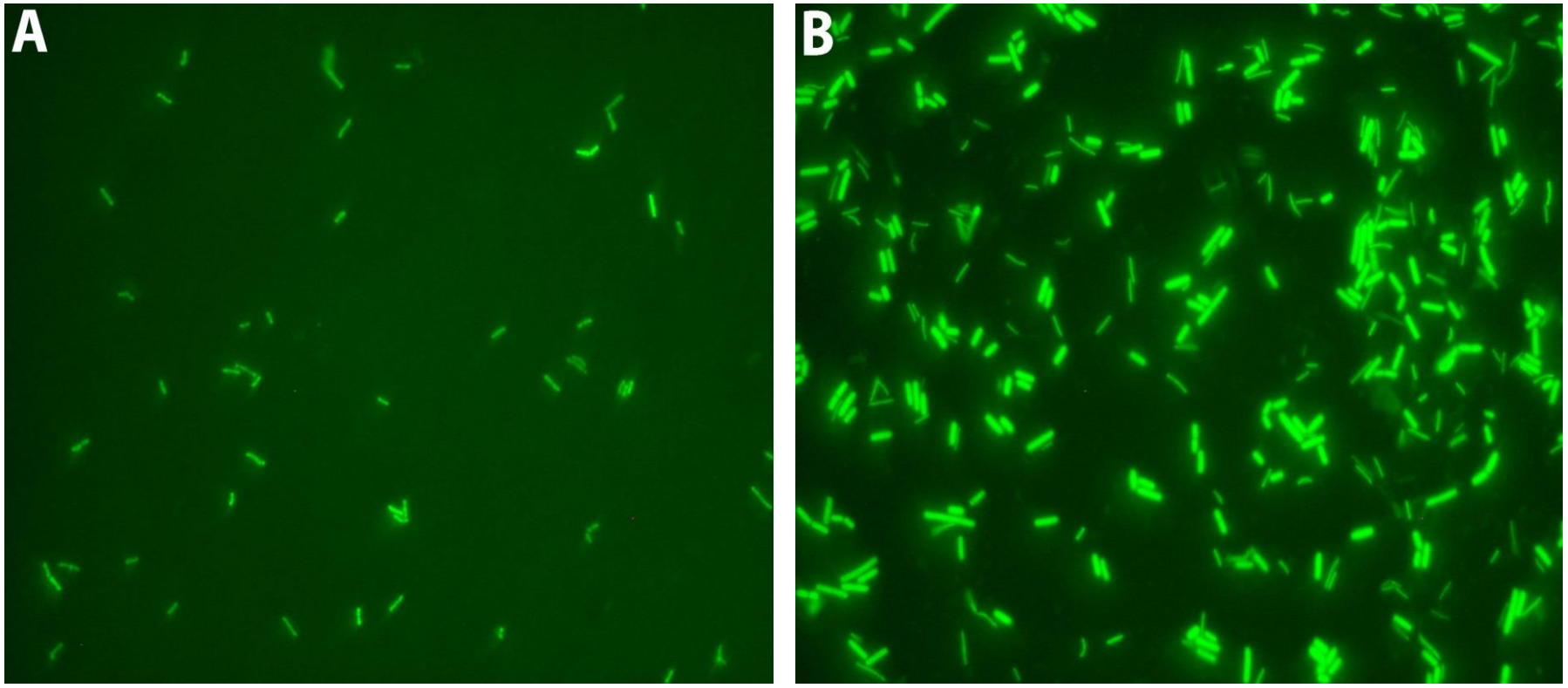
Infant formula

FETUS placental microbiome	**BIRTH** 1. Contact with vaginal-, intestinal-, skin-flora	**BREAST MILK/ INFANT FORMULA**

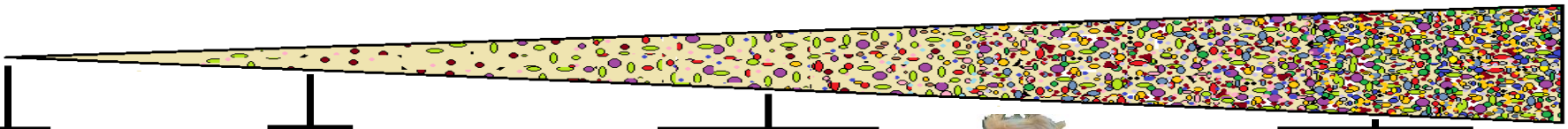

Jakub (vaginal delivery, breast-fed)
TC
Bif
LB
E.coli
Ent
bacterial count in log CFU/g
12
10
8
6
4
2
0
0
50
100
150
200
250
Day after the birth


Kateřina (cesarean section, bottle-fed)
TC
Bif
LB
E. coli
Ent
Bacterial counts in log CFU/g
12
10
8
6
4
2
0
0
50
100
150
200
250
Day after the birth

Infant faeces stained by the FISH procedure using bifidobacteria-specific (A) and clostridia-specific (B) probes



The colonization of the intestine



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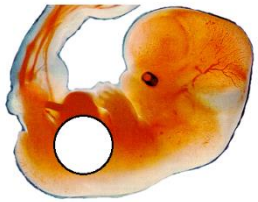

FETUS
placental microbiome

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BIRTH
1. Contact with vaginal-, intestinal-, skin-flora

6 Months

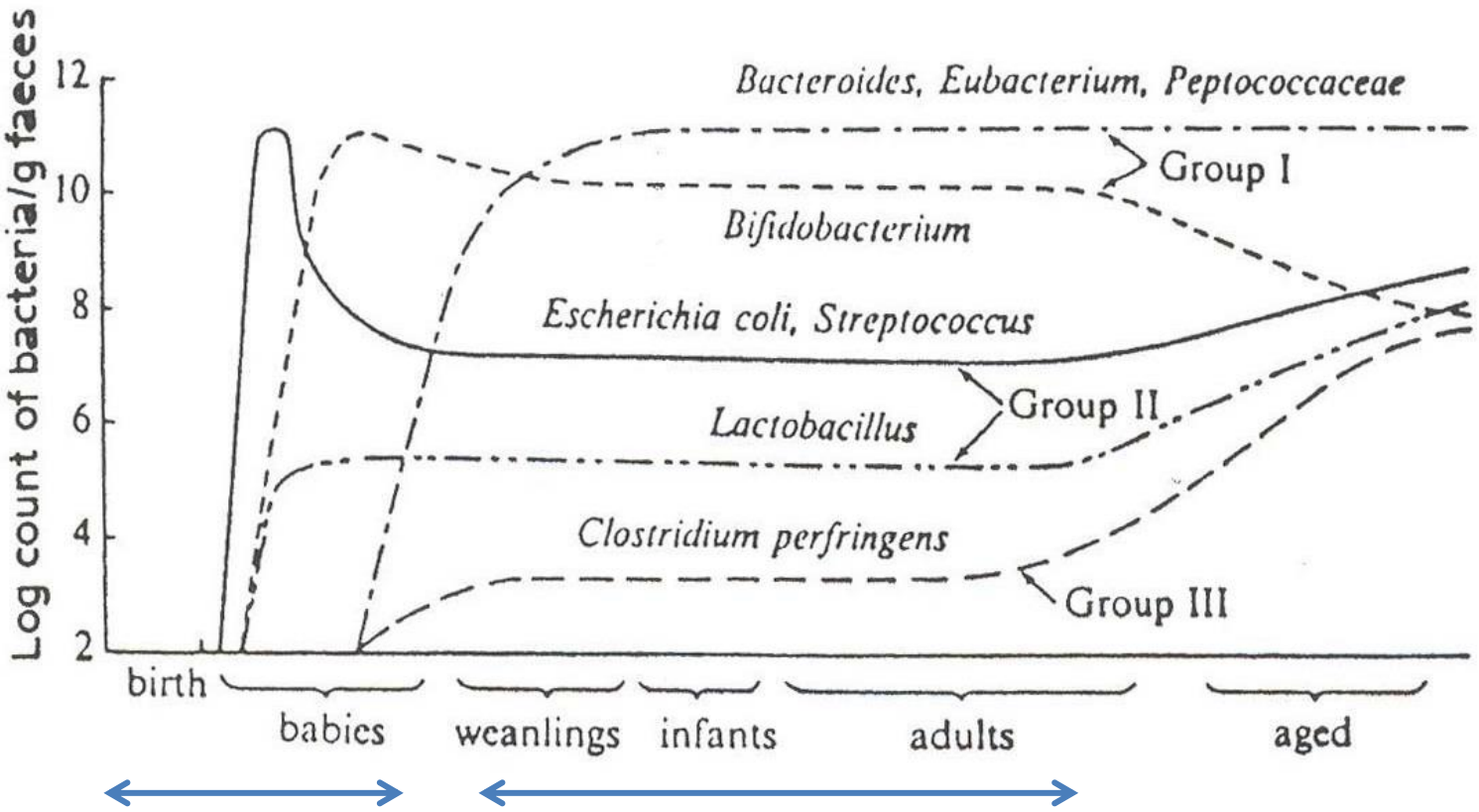
BREAST MILK/ INFANT FORMULA

WEANING
Introduction of solid foods

3 Years

ADULT TYPE of microbiota

Changes in faecal flora during life (Mitsuoka, 1992)

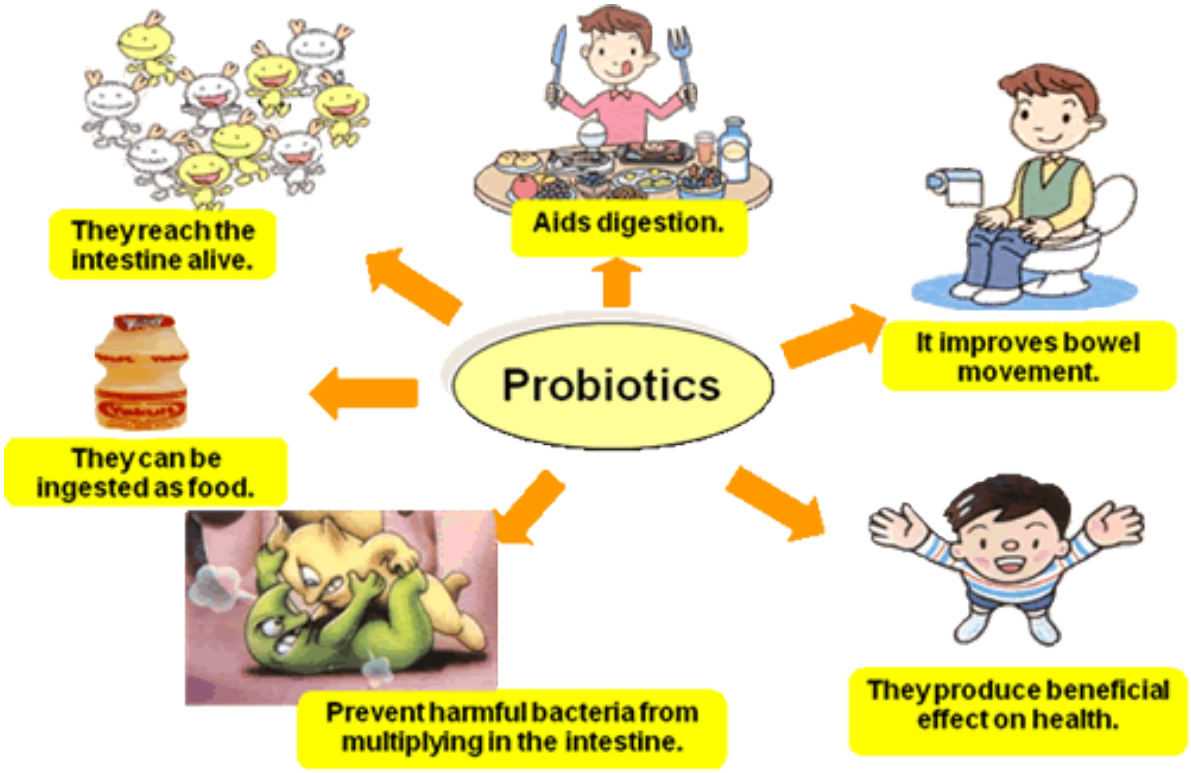


Probiotics **Prebiotics**

Probiotics - definition

„Life microorganisms, which, when administered in adequate amounts confer a health benefit on the host“

(Hill et al., Nat Rev Gastro Hepat 2014)



Probiotic bacteria

Lactic acid bacteria

Lactobacillus acidophilus
Lactobacillus casei
Lactobacillus rhamnosus
Lactobacillus salivarius
Lactobacillus plantarum
Lactobacillus delbrueckii ssp. *bulgaricus*
Lactococcus lactis
Enterococcus faecium
Streptococcus thermophilus
Pediococcus pentosaceus

Bifidobacteria

Bifidobacterium animalis ssp. lactis
Bifidobacterium longum
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium infantis
Bifidobacterium pseudolongum
Bifidobacterium thermophilum

Other bacteria

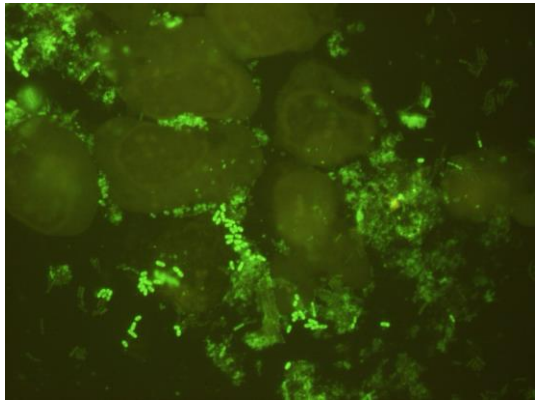
Escherichia coli
Bacillus sp.
Clostridium butyricum

Fungi

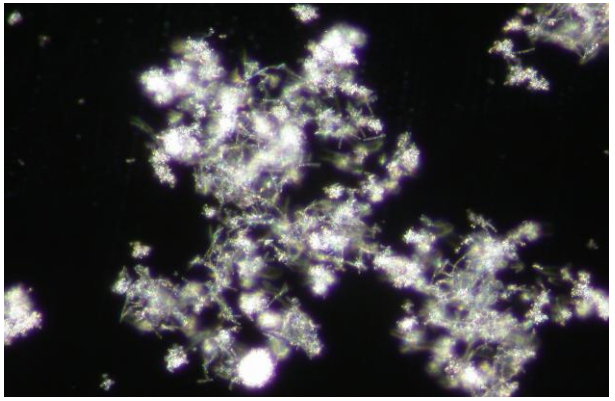
Saccharomyces sp.
Aspergillus oryzae
Candida pintolopesii

Mechanisms of Probiotic Action

- Adhesion to intestinal mucus and epithelium
- Aggregation and coaggregation
- Production of antimicrobial substances
- Nutritional effects
- Prevention and treatment of diarrhea
- (Antitumor effects, treatment/prevention of allergic diseases, inflammatory bowel diseases, infection of respiratory tract, etc.)



Adherence to epithelial cells (Kmeť, 2004)



Coaggregation

Nutritional effects

- Alleviation of lactose intolerance symptoms (ß-galactosidase; approved by EFSA)
- Production of vitamins (B, K)
- Reduction of serum cholesterol

Production of antimicrobial substances

- Organic acids
- Hydrogen peroxide
- Bacteriocins (nisin E234, sakacin)
- Reuterin

Prevention and treatment of diarrhea

- Antibiotic-associated diarrhea (*Saccharomyces boulardii*)
- Traveler´s diarrhea (prevention)
- *Clostridium difficile*-associated diarrhea (stool transplantation)

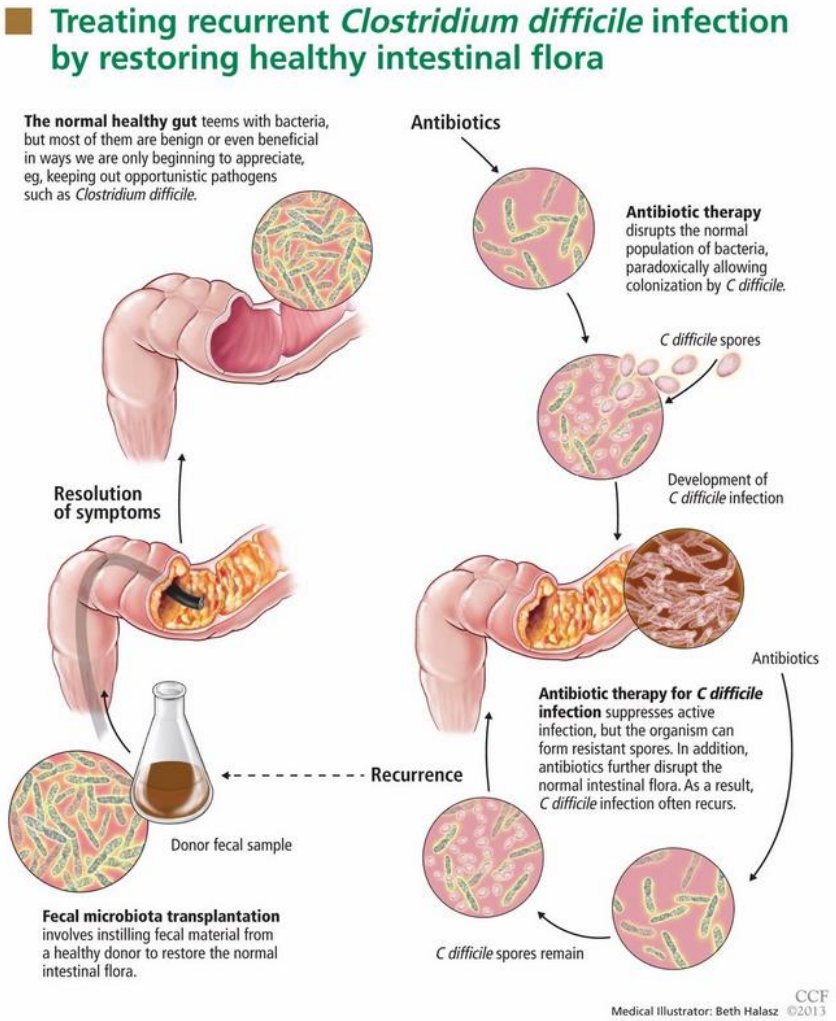
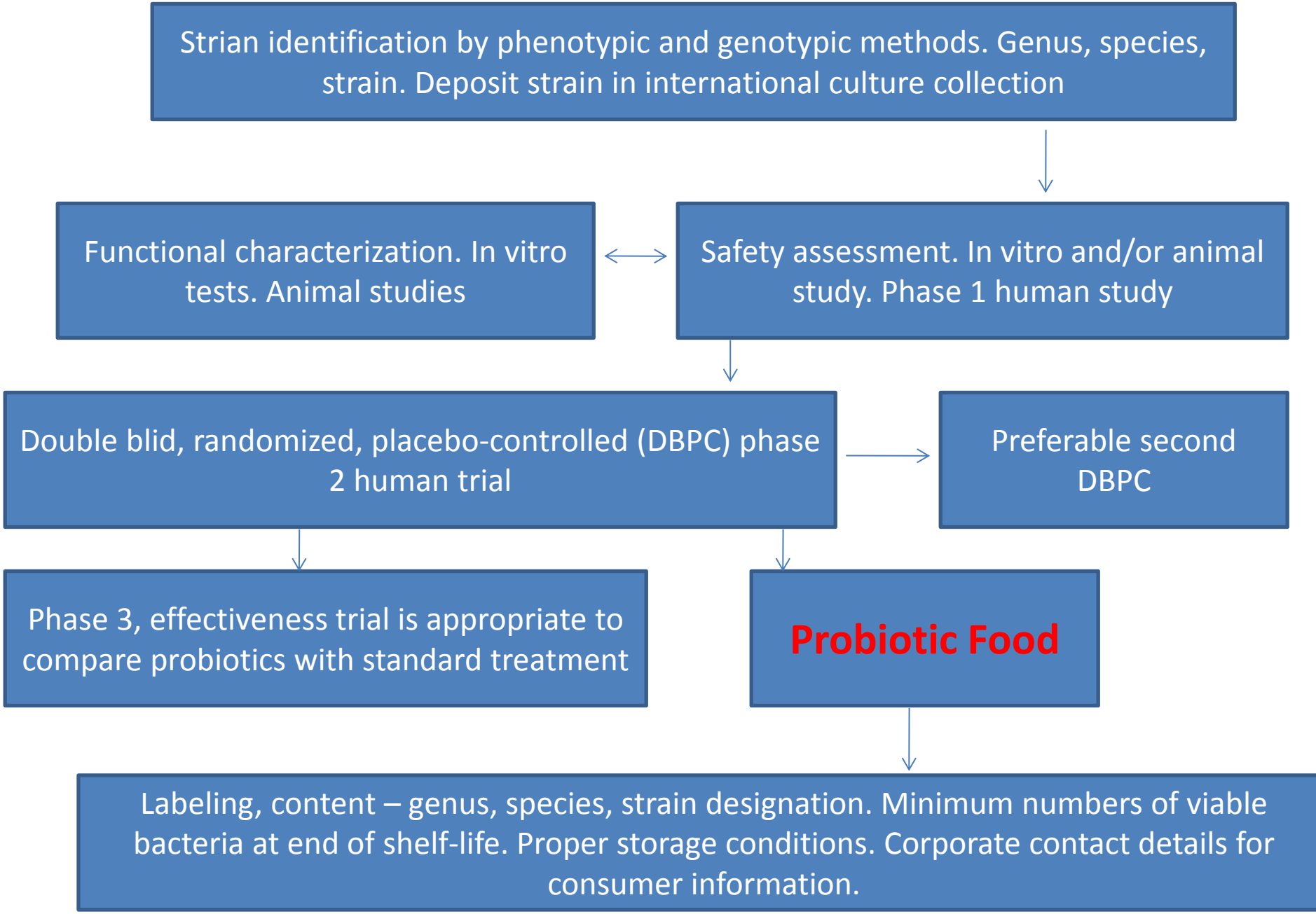


FIGURE 1.

Guidelines for the evaluation of Probiotics for Food Use (FAO/WHO 2002)



Commercially available probitic organisms

- *Lactobacillus acidophillus* LA5 (Chr. Hansen)
- *Lactobacillus rhamnosus* GG (LGG; ATCC 53103; Gefilus®)
- *Lactobacillus casei* Shirota (Yakult)
- *Lactobacillus casei* Imunitass (Danone)

- *Bifidobacterium animalis* subsp. *lactis* DN173010 (Danone)
- *Bifidobacterium animalis* subsp. *lactis* BB 12 (Chr. Hansen)
- *Bifidobacterium longum* BB536 (Murinaga)
- *Bifidobacterium breve* (Yakult)

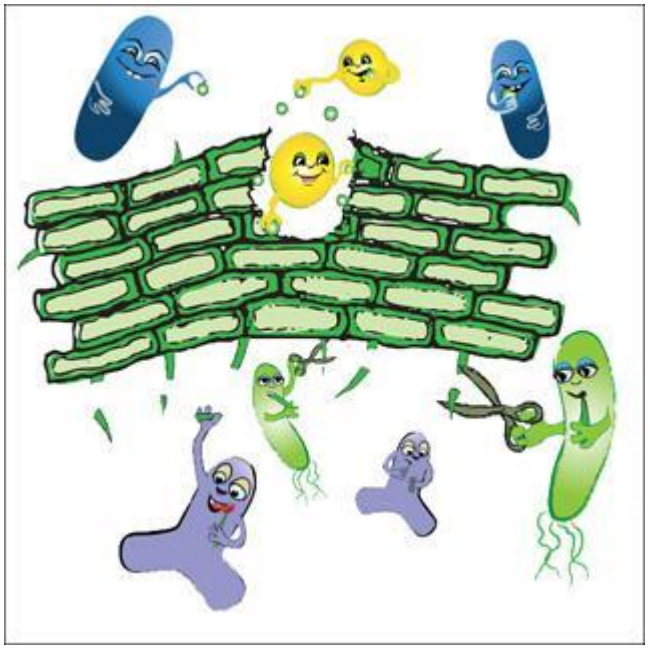
Commercially available probitic organisms

Effective dosage for probiotic effect?

- Daily dose (10^7- 10^{10} CFU)
- Daily frequency of administration (1-4 times)
- Timing of administration
- Duration of administration (1day – several months)
- Method of delivery (fermented food, beverages, capsule, tablet)
- Viability

Prebiotics - definition

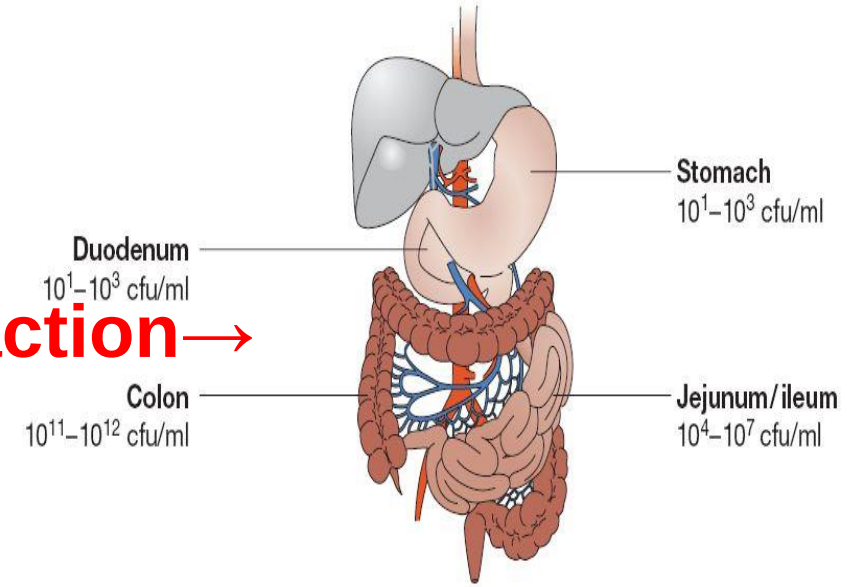
„Nondigestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health“ (Gibson and Roberfroid, 1995) (Inulin, soya oligosaccharides)



Criteria for Prebiotics (Roberfroid, 2007)

- resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption
- fermentation by intestinal microbiota
- selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being

Prebiotic action→



Prebiotics

- FOS – fructooligosaccharides
- GOS – galactooligosaccharides
- SOS – soya oligosaccharides
- XOS – xylooligosaccharides
- MOS – isomaltooligosaccharides
- HMOs – human milk oligosaccharides

Inulin content in selected plants (Ebringer, 2002)

Plant	inulin (FOS) content in g/100g
Onion	2 – 7
Garlic	9 – 16
Leek	3 – 10
Sweet potatoes	13 – 20
Jerusalem artychoke	16 – 40
Dandelion	12 – 15
Banana	0.3 – 0.7