



Basics facts about the human microbiota

MUDr. Jakub Hurych

Department of Medical Microbiology, Second Faculty of Medicine, Charles University, Prague



Microbiome vs. microbiota



Microbiome = set of microbial genomes at given site

Microbiota = set of microorganisms present in an environmental habitat

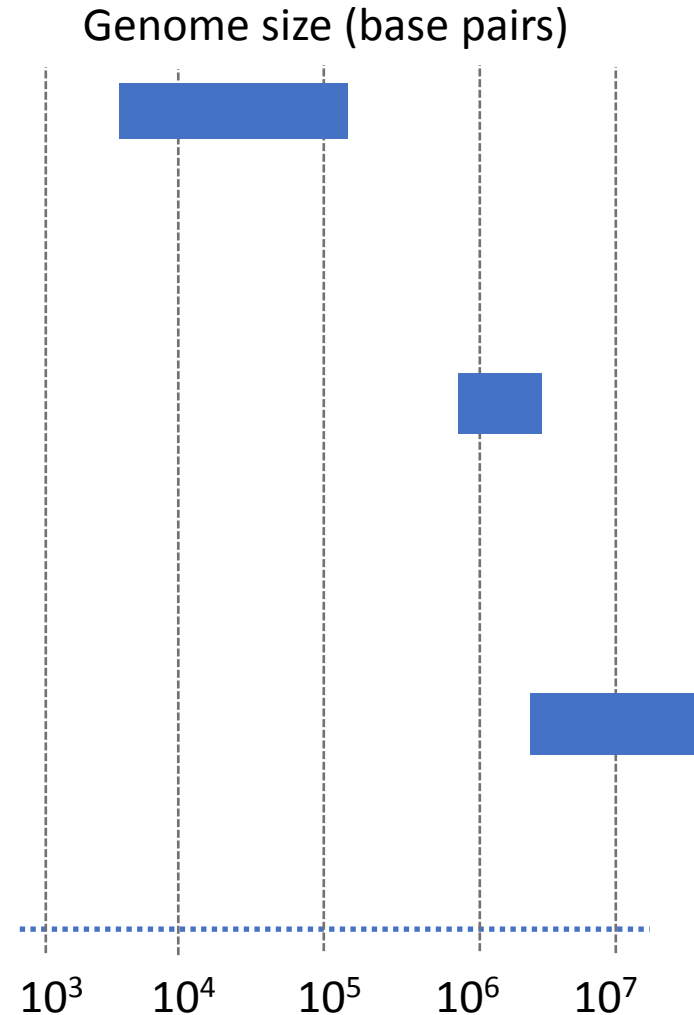
Structure of the lecture

1. How to investigate the humane microbiome
2. Structure and function of the healthy adult human microbiota
3. Development of the human microbiota
4. Disorders attributed to the human microbiota
5. Modulation of the human microbiota

Microbiome

Set of all microbial genomes at given site

- **Viruses** (virome)
 - smallest genome
 - very variable content in the microbiome
 - **no shared sequence across viruses**
- **Bacteria** (bacteriome)
 - intermediate genome
 - subject of most published works
 - **shared sequences in ribosomal DNA**
- **Fungi** (mycobiome)
 - eukaryotes
 - 50+ times bigger than bacteria
 - 0,1% - 1% of the gut microbiome
 - shared sequences in ribosomal DNA

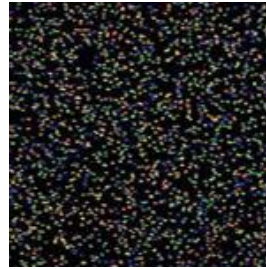


Massive parallel sequencing

one **fragment** of DNA



one **cluster** (dot)
on the sequencing chip



one **read** in the resulting file

```
@M01641:32:000000000-AFYJ6:1:1107:20897:19055 2:N:0:1
CGCGGATGGAGGAGATGGGCAGCCGACCCGCGACCGACTGGACGAGGTGAAG
GAGCAGGTGGCGGAGGTGCGCGCCAAGCTGGAGGAGCAGGCCAGCAGATACG
CCTGCAGGCCGAGGCCTCCAGGCC
+
CCCCDBBBFFBEGGGGGGGGGHGGGGGGGGGGGGGGHHHE@EGEHGHH
GGHHHHHHHGGFGDHHGGGGDC EFHHHHGGGGHHHGEDFHFHGHBBGFG
GCGHHFHGGG?AGGGFGGGGGGFGG
```

100,000 – 5 billion **fragments**

Methods of microbiome investigation

Amplicon profiling: what is there?



DNA extraction

Metagenomic sequencing

Generic PCR
for **16S rDNA**



Perform mass sequencing
of this gene



Group reads by
similarity, count them

>OTU1: AAGCATATGCTATGATCGATCATGACT
>OTU2: CATGATCTGACTATTATTCGCGATTG
>OTU3: GCGATATTCGATCTATTCGATGCGGAT
.....

Classify taxonomy

>OTU1: Firmicutes; Clostridia; Clostridium piliforme
>OTU2: Firmicutes; Clostridia; Ruminococcus bromii
>OTU3: Firmicutes; Bacilli; Leuconostoc
.....

Randomly fragment
the **total DNA**

Sequence,
assemble genes



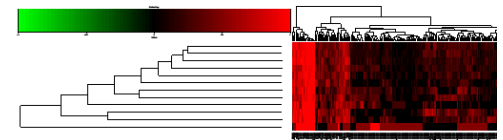
Classify
genes
by origin

Classify genes
by function

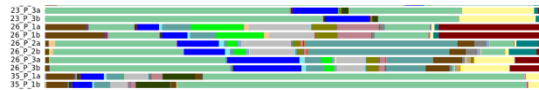
> glucosidase
> lambda phage capsid
> lactase
> cable pilus
.....

Assess **functional capabilities** of
the microbiome

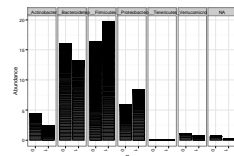
Compare cases with
controls



Analyze composition



Compare cases with
controls



Methods of microbiome investigation

Amplicon profiling:
what is there?

100-250 bases



DNA extraction

Metagenomic sequencing

Whole genomes

Generic PCR for 16S rDNA

Less costly, more samples.

Perform mass sequencing of this gene

Group reads by similarity

Only bacteria or fungi, no viruses.

Classify it

>OTU1: AAGCATATGCTATGATCGATCATGACT
>OTU2: CATGATGTGACTATTTCGCGATTTC
.....
>OTU4: Firmicutes; Clostridia; Clostridiales
>OTU5: Firmicutes; Clostridia; Clostridiales
>OTU3: Firmicutes; Bacilli; Leuconostoc
.....

Randomly fragment the **total DNA**

Sequence, assemble



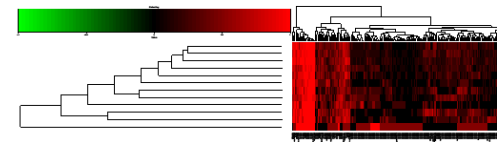
Classify genes by origin

Classify genes by function

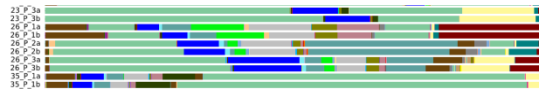
- > glucosidase
- > lambda phage capsid
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-

Assess **functional capabilities** of the microbiome

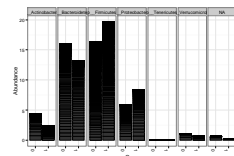
Compare cases with controls



Analyze composition



Compare cases with controls



Microbiome

IN NUMBERS

100 Trillion

symbiotic microbes live in and on every person and make up the human microbiota

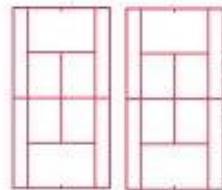
The human body has more microbes than there are stars in the milky way

95%

of our microbiota is located in the GI tract

150:1

The genes in your microbiome outnumber the genes in our genome by about 150 to one



The surface area of the **GI tract** is the same size as 2 tennis courts

You have 1.3X

more microbes than human cells

>10,000

Number of different microbial species that researchers have identified living in and on the human body

2kg

The gut microbiota can weigh up to 2Kg



Microbiome Ireland

Interfacing Food & Medicine

The microbiome is more medically accessible and manipulable than the human genome

90%

It is thought that 90% of disease can be linked in some way back to the gut and health of the microbiome

5:1

Viruses:Bacteria in the gut microbiota



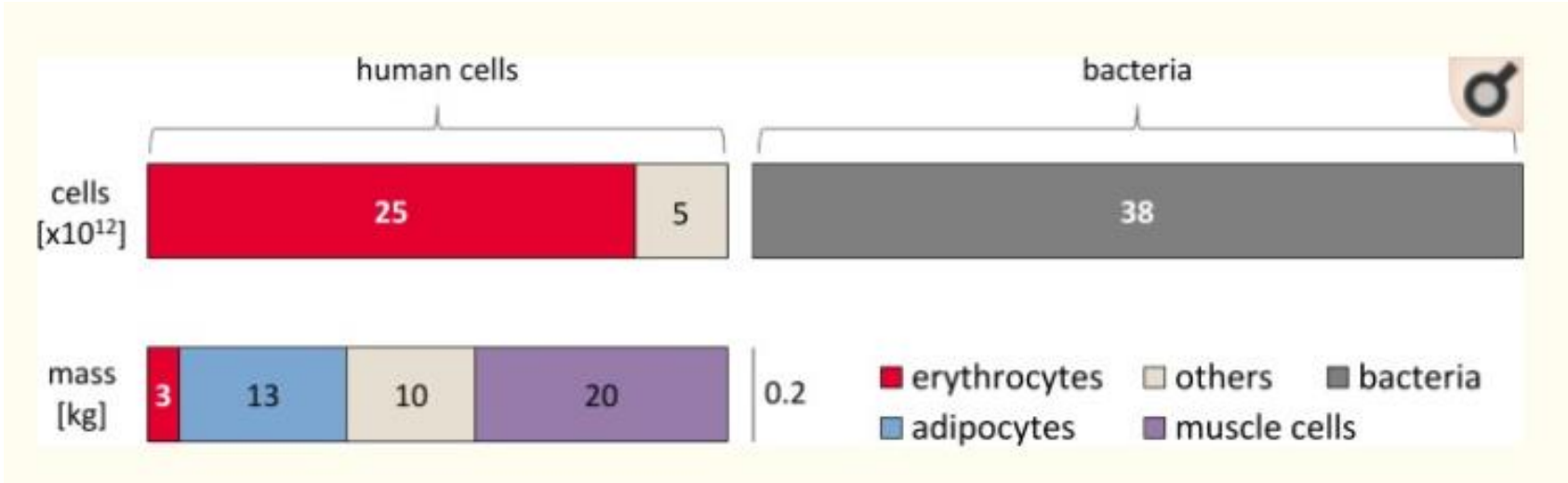
2.5

The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint



We are outnumbered by microbes



Sender et al, PLOS, 2016

	Cell count	Genes count	Mass
Human	30 trillion (3.0×10^{13})	20-25 thousand (2.0×10^4)	70-100 kg
Microbes	38 trillion (3.8×10^{13})	2-20 milion ($2.0 \times 10^6 - 2.0 \times 10^7$)	1-3 kg
	1.3 x more microbial	100x more microbial	Still more human



What is their role?

1) GI tract

- Digestion and absorption of nutrients
- Nutrition for enterocytes – productin of SCFA
- Gut lineage integrity

2) Metabolism

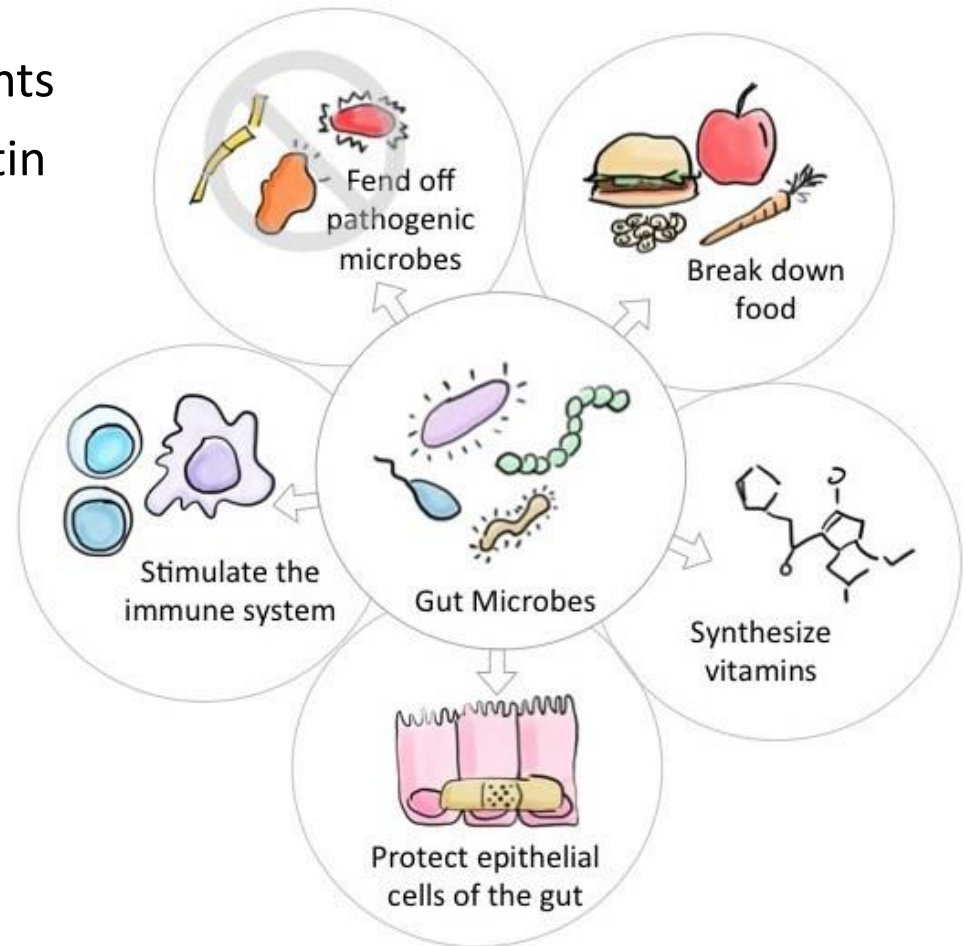
- pH stability, metabolite productions

3) Immunity

- Immune system maturation
- Prevention of infection

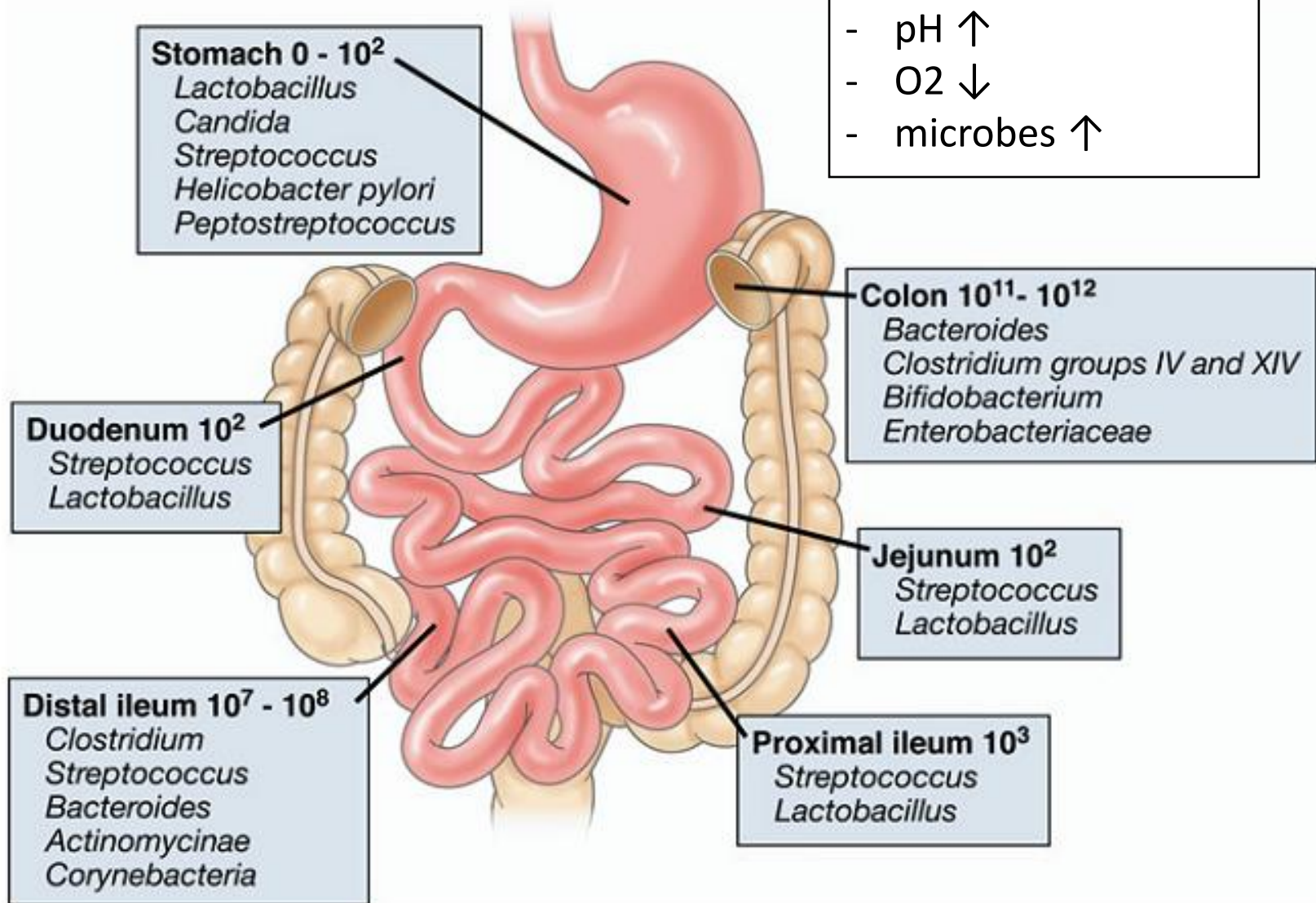
4) Epigenetics

Influencing the pathogenesis of diseases



The gut microbiota

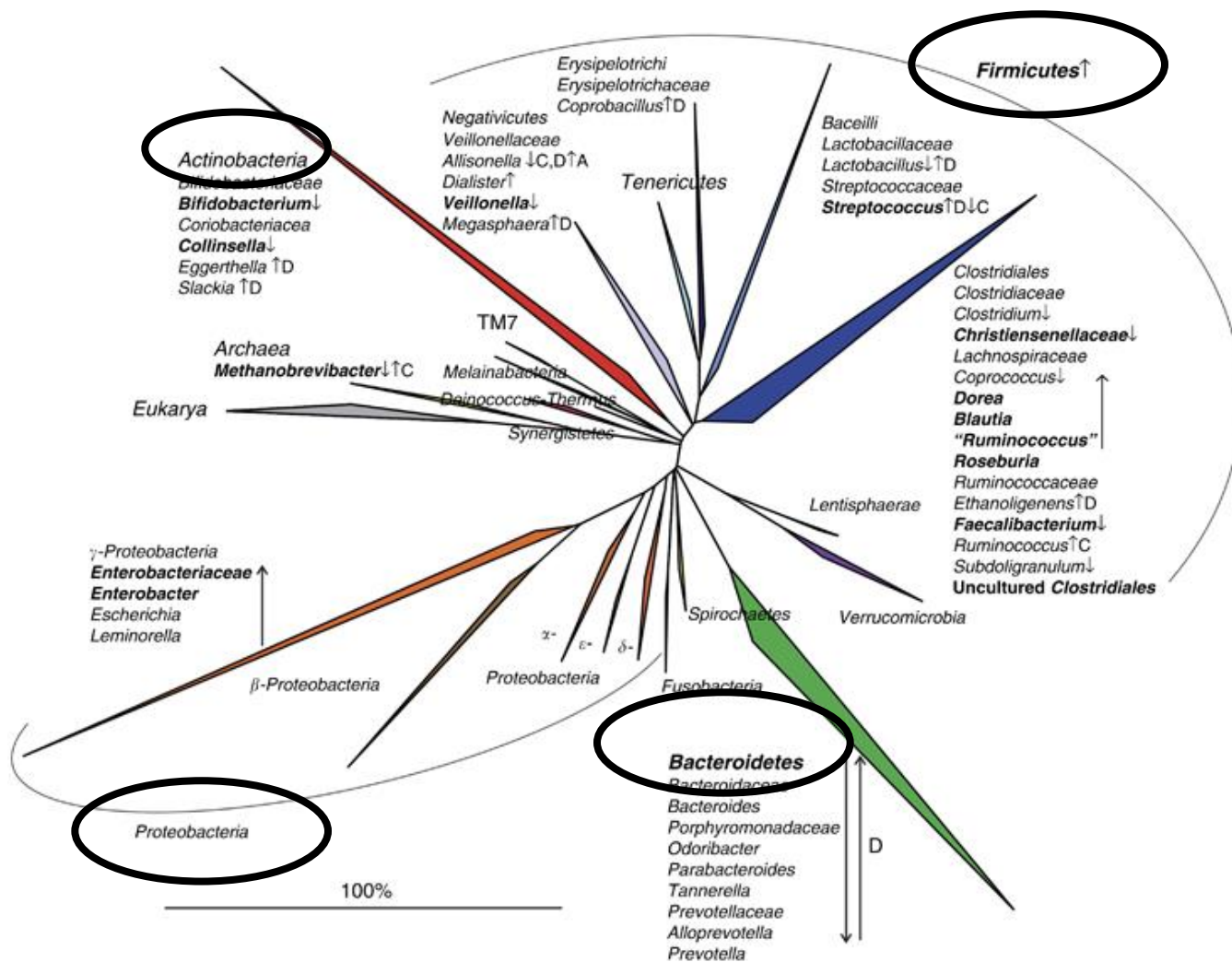
Where do they live?



The more distant:

- pH ↑
- O₂ ↓
- microbes ↑

Who they are?



Our guests

Main Phyla	Class	Example Genera
<i>Actinobacteria</i>	<i>Actinobacteria</i>	<i>Actinomyces; Bifidobacterium</i>
<i>Bacteroidetes</i>	<i>Bacteroidia</i>	<i>Bacteroides; Prevotella; Alistipes</i>
<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillus; Staphylococcus</i> <i>Enterococcus; Lactobacillus;</i> <i>Lactococcus; Streptococcus;</i> <i>Leuconostoc</i>
	<i>Clostridia</i>	<i>Clostridium; Coprococcus;</i> <i>Roseburia; Faecalibacterium;</i> <i>Ruminococcus</i>
	<i>Negativicutes</i>	<i>Veillonella</i>
<i>Proteobacteria</i>	<i>Epsilonproteobacteria</i>	<i>Helicobacter; Campylobacter</i>
	<i>Gammaproteobacteria</i>	<i>Citrobacter; Escherichia; Shigella;</i> <i>Klebsiella; Providencia</i>
<i>Verrucomicrobia</i>	<i>Verrucomicrobiae</i>	<i>Akkermansia</i>

What do they eat?

	g/day
• Our diet	
• resistant starch (i.e., to amylase)	8-40
• polysaccharides (pectins, glucans, etc)	8-40
• unabsorbed sugars and alcohols	2-10
• oligosaccharides	2-8
• proteins	3-9
• Our secretions	
• mucus (glycoproteins)	2-3
• proteins (enzymes, antimicrobial peptides)	4-6
• Our cells	20-30

What do they produce?

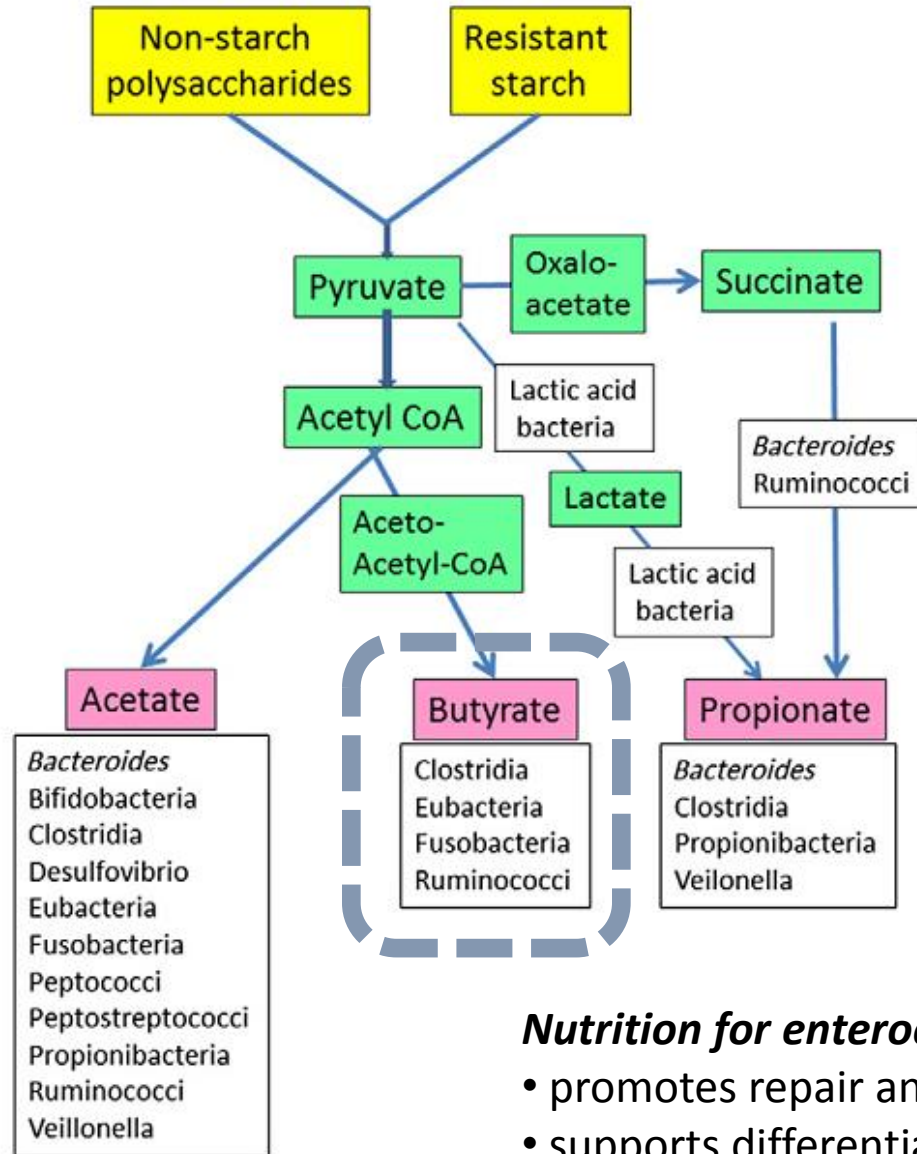
Capacity comparable to that of **liver**

- **Vitamins**

- metaquinones, a bacterial form of vitamin K
- vitamin B12 – probably not utilized
- other B vitamins

- **Short chain fatty acids**

Short chain fatty acids (SCFA)



Nutrition for enterocytes:

- promotes repair and proliferation,
- supports differentiation,
- tightens the junctions

Why are SCFA important?

Processing of undigestive material

SCFA production

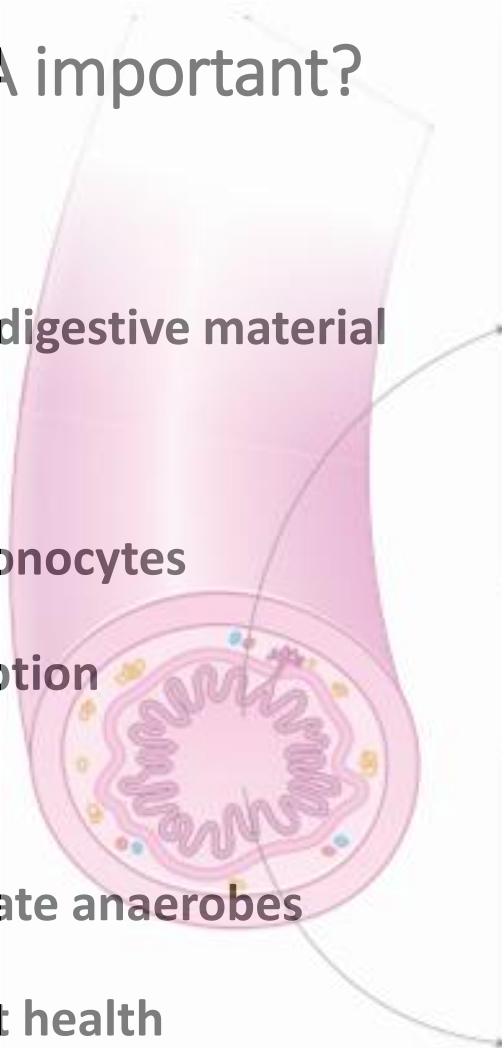
Nutrition for colonocytes

Oxygen consumption

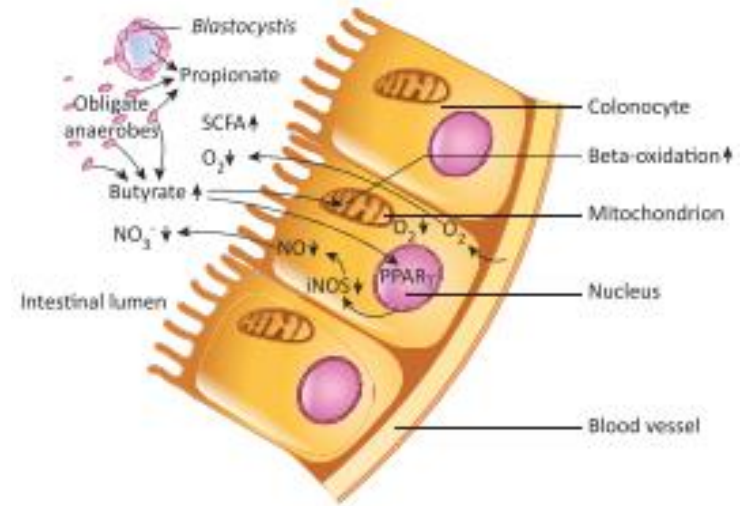
Anaerobic state

Thriving of obligate anaerobes

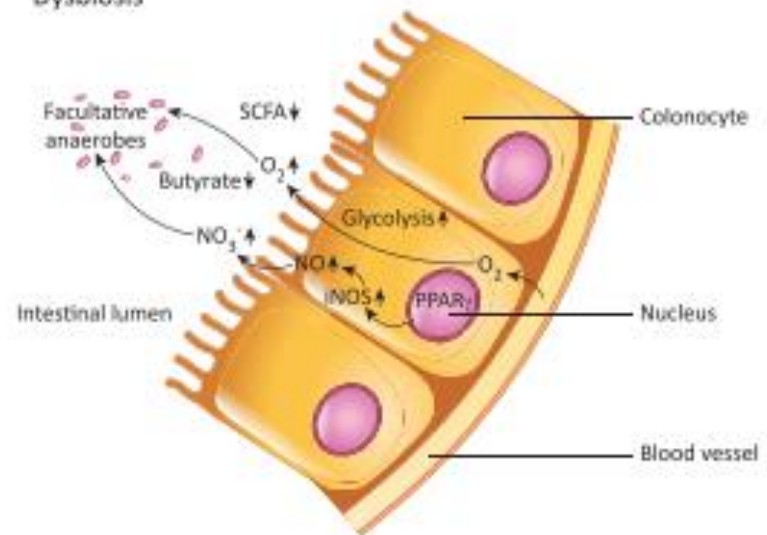
Promotion of gut health



Eubiosis



Dysbiosis

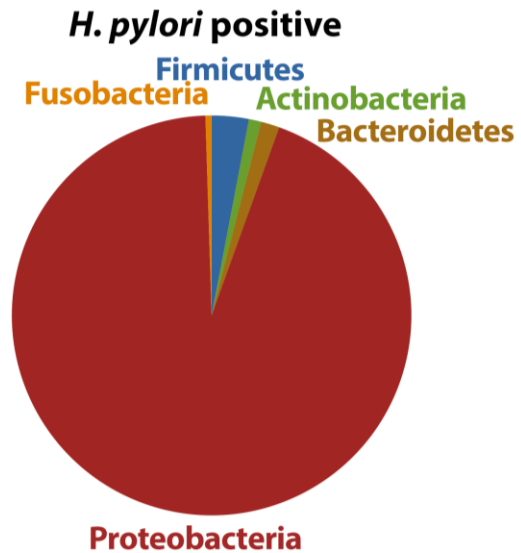
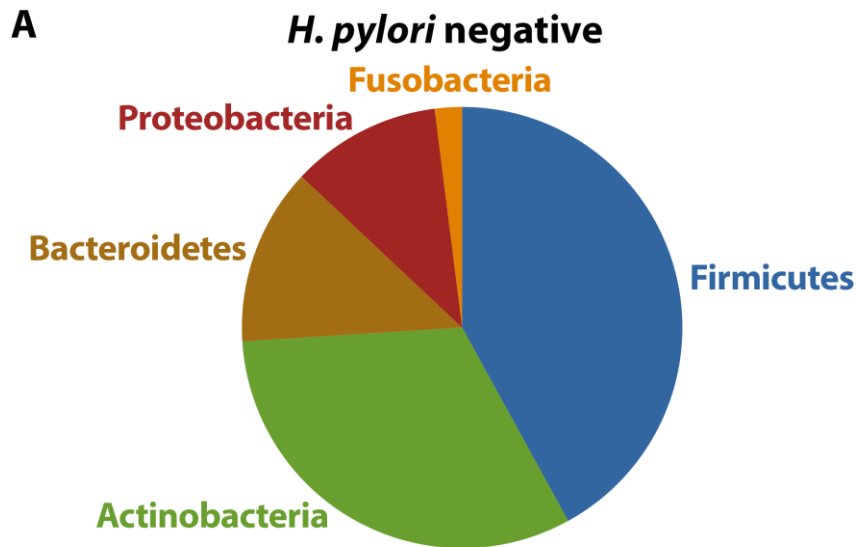


Stomach

Stomach is not sterile

H. pylori

- 50% of population colonised
- When presented – 20% risk of GI disorder
- Class I carcinogen in adults, **protective in children**



Small intestine

Duodenum

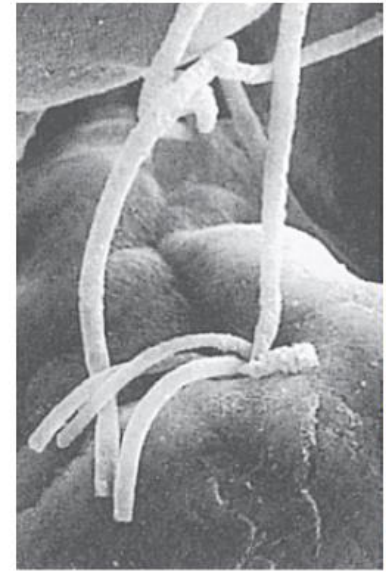
- Acidic environment

Jejunum and ileum

- pH gradually increases as well as the count of microbial cells (10^5 - 10^7 /g)
- Microbiota more anoxic,
- Digest small carbohydrates



(a)



(b)

Large intestine

The gut microbiome – 99% formed by *Bacteria*

- **Mostly obligate *anaerobes***
- Enormous species diversity: 3,500 - 35,000
- Main phyla
 - *Actinobacteria*
 - ***Bacteroidetes***
 - ***Firmicutes***
 - *Proteobacteria*

Other 1%: *Archea* and yeasts

- ***Methanobrevibacter smithii*** – reduces the gas formation
- *Candida*

Enterotypes



Proteolytic bacteria:

Bacteroides, *Streptococcus*,
Staphylococcus, *Proteus*, *Escherichia*,
ome species of *Clostridium*, *Fusobacteria*,
Bacillus, *Propionibacterium*...



Saccharolytic bacteria

Prevotella, *Bifidobacterium*, *Lactobacillus*,
Eubacterium, *Propionibacterium*,
Escherichia, *Enterococcus*,
Peptostreptococcus, *Fusobacteria*...

Wu et al. Science 2011

Enterotype = classification of living organisms based on its bacteriological ecosystem in the gut microbiome

- Not influenced by age, gender, body weight, or national divisions
- Influenced by long-term diet
- Part of the population structure (?)

Enterotype I

Bacteroides predominant

Enterotype II

Prevotella predominant

Gut brain axis

Is autism (ASD) connected to the dysbiosis?

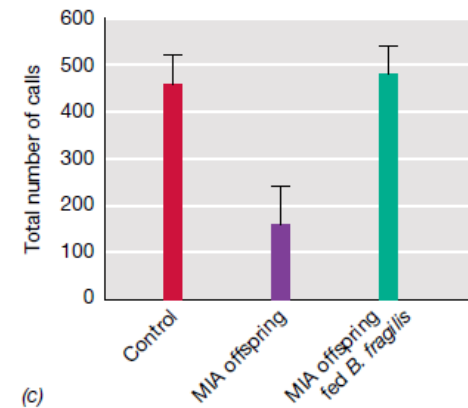
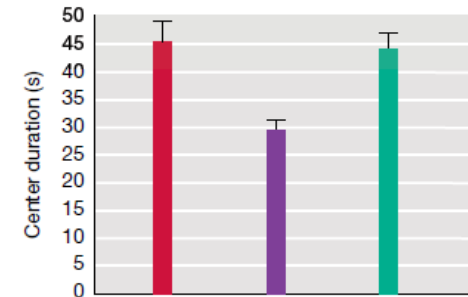
- loss of intestinal integrity
- a shift in the composition of gut clostridial and bacteroidal populations
- 46-fold increase in serum levels of the chemical **4-ethylphenylsulfate**



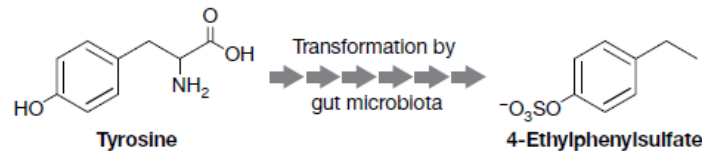
(a) Reduced exploration



(b) Reduced vocalization



(c)

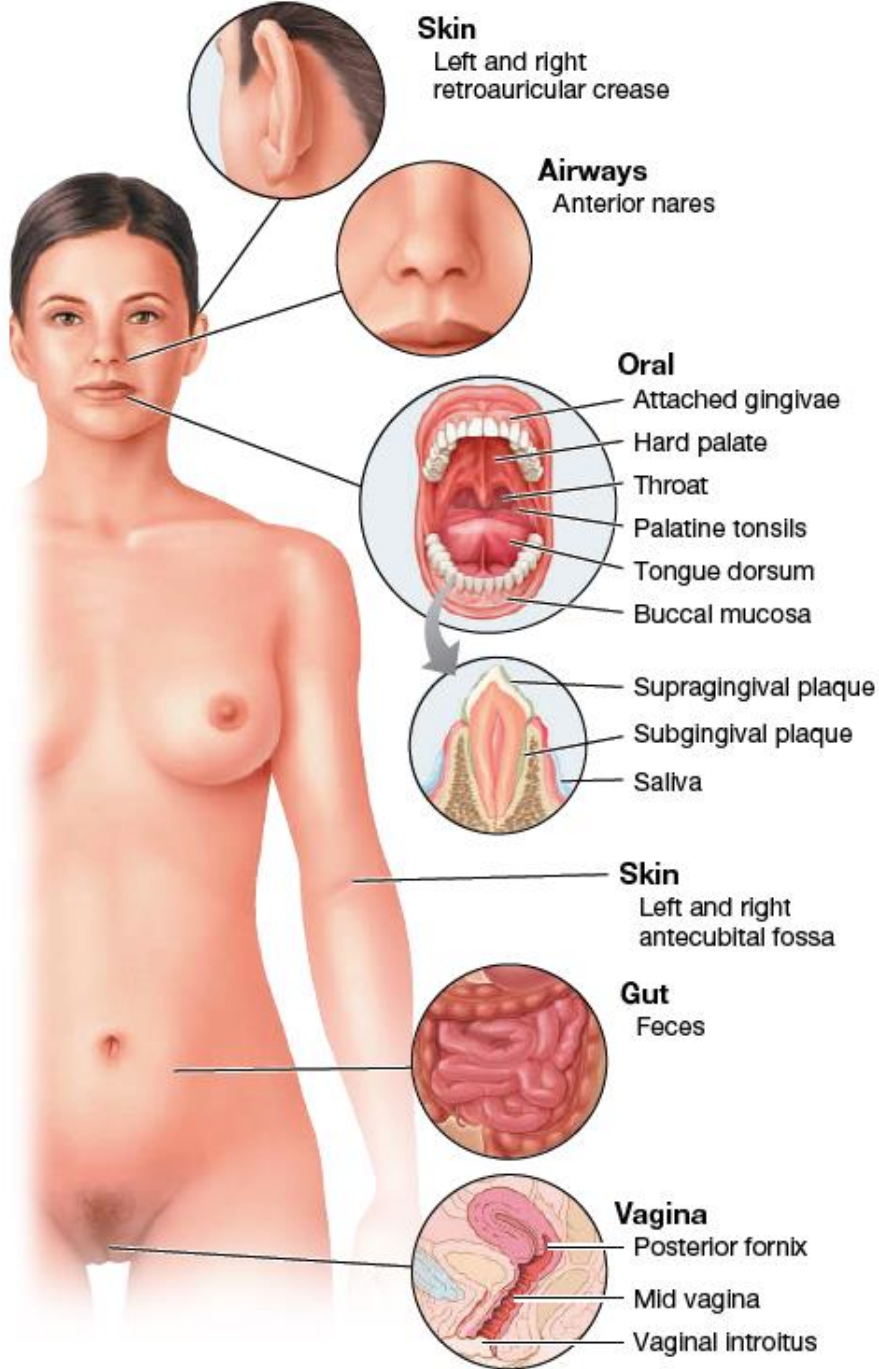


(d)

Figure 1 Influence of gut microbiota on behavior. Mice offspring of mothers with maternal immune activation (MIA) show autistic-like behavior, marked by (a) a fear of exploring the center of the test field and (b) reduced vocalization. (c) Feeding the affected offspring the human commensal bacterium *Bacteroides fragilis* ameliorates these behavioral abnormalities. (d) Certain gut microbiota can convert the amino acid tryptophan into 4-ethylphenylsulfate, the neuroactive compound thought to trigger the mouse autistic-like behaviors shown in a and b. Modified from Hsiao, E.Y., et al. 2013. *Cell* 155: 1451–1463.

Other inhabited sites

- Mouth
- Nasal cavities
- Throat
- Stomach
- Intestines
- Urogenital tracts
- Skin



Oral cavity

Mucin in mucus inhibits microbial attachment

When the teeth appear – colonization by anaerobes making biofilms
(*Streptococcus*)

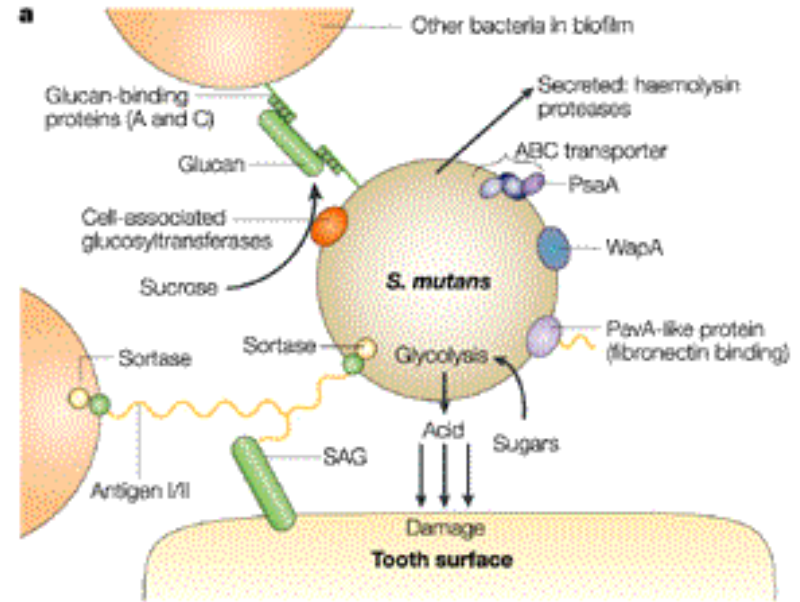
Tooth decay, gum inflammation,
and periodontal disease – manifestations
of **oral dysbiosis**



Oral cavity

As diverse as the gut, but less interindividually

- Mostly facultative aerobic, some obligately anaerobic
- *Veillonella parvula* – most abundant single species
- ***Streptococcus*** – most abundant genus (up to 25% of all)
- *S. mutans* – tooth decay



Phylum	Families, Genera and Species
<i>Firmicutes</i>	<i>Streptococcus</i>, <i>Veillonella</i>, <i>Peptostreptococcus</i>,
<i>Bacteroidetes</i>	<i>Bacteroides</i>, <i>Prevotella</i>, <i>Porphyromonas</i>
<i>Proteobacteria</i>	<i>Neisseria</i>

Upper respiratory tract

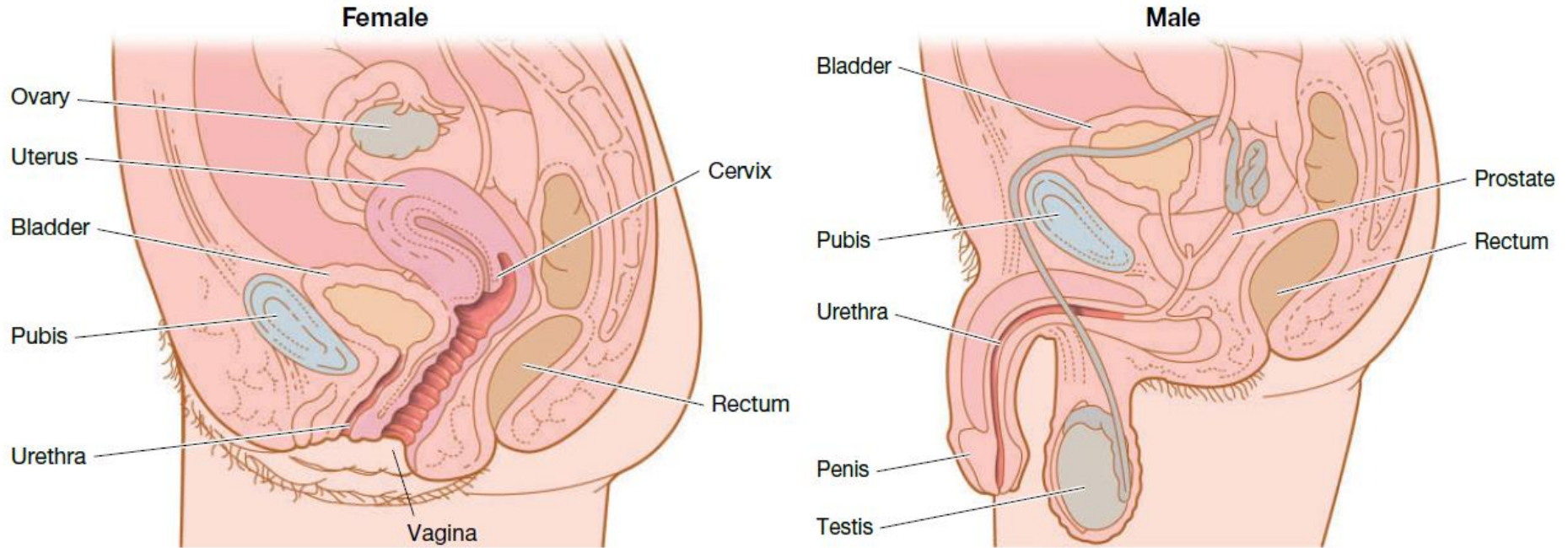
- Mostly transient bacteria
- Few colonizers: coagulase-negative staphylococci, streptococci, diptheroid bacilli, gram-negative cocci (*Neisseria* spp.), *Moraxella* (nose)
- Potential pathogens such as *S. aureus* and *S. pneumoniae* occasionally part of the normal microbiota in the nasopharynx of healthy individuals

Phylum	Families, Genera and Species
<i>Firmicutes</i>	<i>Streptococcus</i>, <i>Veillonella</i>, <i>Peptostreptococcus</i>, <i>Propionibacterium</i>
<i>Actinobacteria</i>	<i>Propionibacterium</i>
<i>Proteobacteria</i>	<i>Neisseria</i> , <i>Moraxella</i>
<i>Bacteroidetes</i>	

Lower respiratory tract

- **Dynamic microbiota** – „no“ residential microbes in healthy adults
- Only particles smaller than 10 μm in diameter could reach the lungs – may include some possible pathogenic bacteria

Urogenital tract



Kidneys, urethers and bladder are **sterile**

Distal urethra, vagina and penis colonised

Distal urethra

- Mostly facultatively aerobic gram-negative bacteria, coagulase-negative staphylococci, diptheroid bacilli, enterococci
- Potential pathogens as *E.coli* and *Proteus mirabilis*

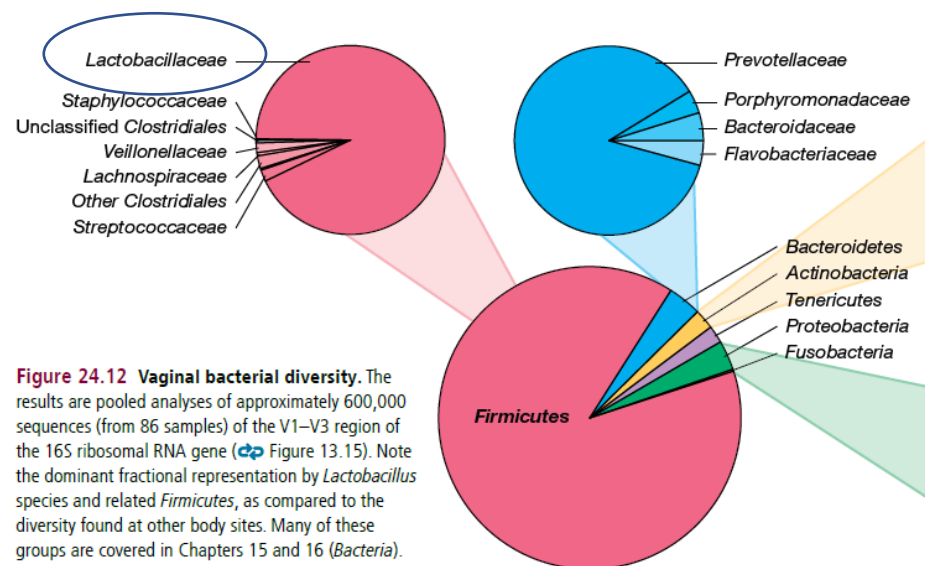
Vaginal microbiota

Lower diversity (same in penis)

Healthy adult vaginal microbiota dominated by **lactobacilli**

- Absent before puberty, after that present and makes acidic environment
- Five types of vaginal communities

Other microbes include streptococci, coagulase-negative bacteria, corynebacteria, diptheroids, *Candida*



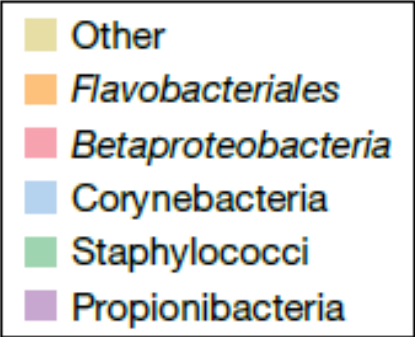
Skin microbes

Ca. 1 million resident bacteria/cm²

Mainly three genera: *Corynebacterium*, *Propionibacterium*, *Staphylococcus*

Specifics in each microenvironment

- Moist sites: corynebacteria (diphtheroids), staphylococci
- Drier sites: *Betaproteobacteria*, *Flavobacteriales*
- Sebaceous glands: *Propionibacterium*



(b)

Other aspects of the skin microbiota

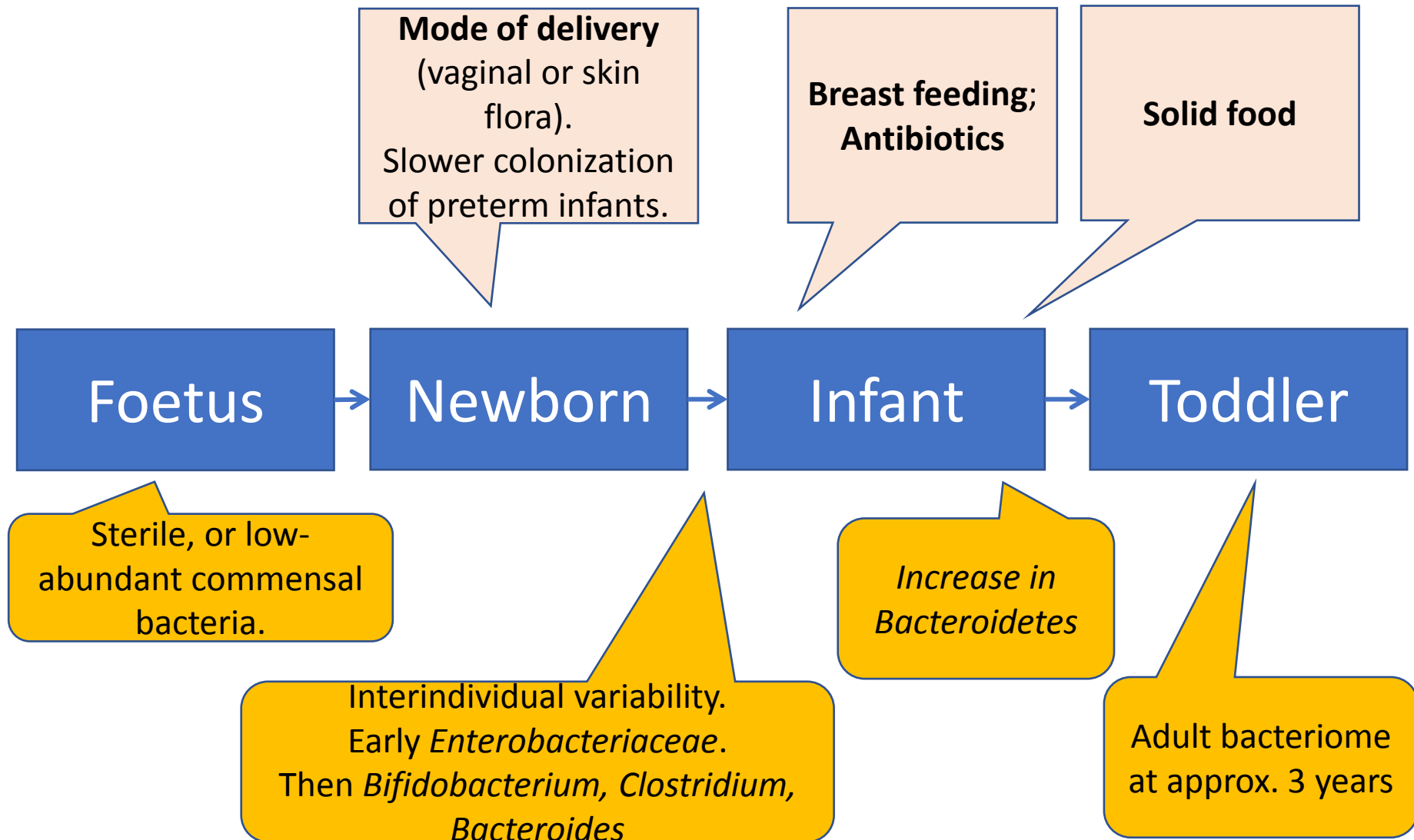
Not only Bacteria – also Archea and Fungi

- *Malassezia* – most common fungi
- *Candida* in immunocompromised patients

Factors influencing the skin microbiota:

- **Weather** – increasing/decreasing moisture and temperature
- **Age** – more varied in children, microbiome transition with sexual maturation
 - Young children: *Streptococcus spp.*, *Betaproteobacteria* and *Gammaproteobacteria*
 - Postadolescent young adults: *Propionibacterium* and *Corynebacterium*
- **Personal hygiene** – the more rigid, the less diverse microbiota

Development of the microbiota



Dysbiosis

- Disturbance of the microbiota from what is considered normal
- Most connected disorders:
 - **Obesity**
 - **Inflammatory bowel disease**
 - Colorectal cancer
 - Dental caries and periodontitis
 - Acne vulgaris

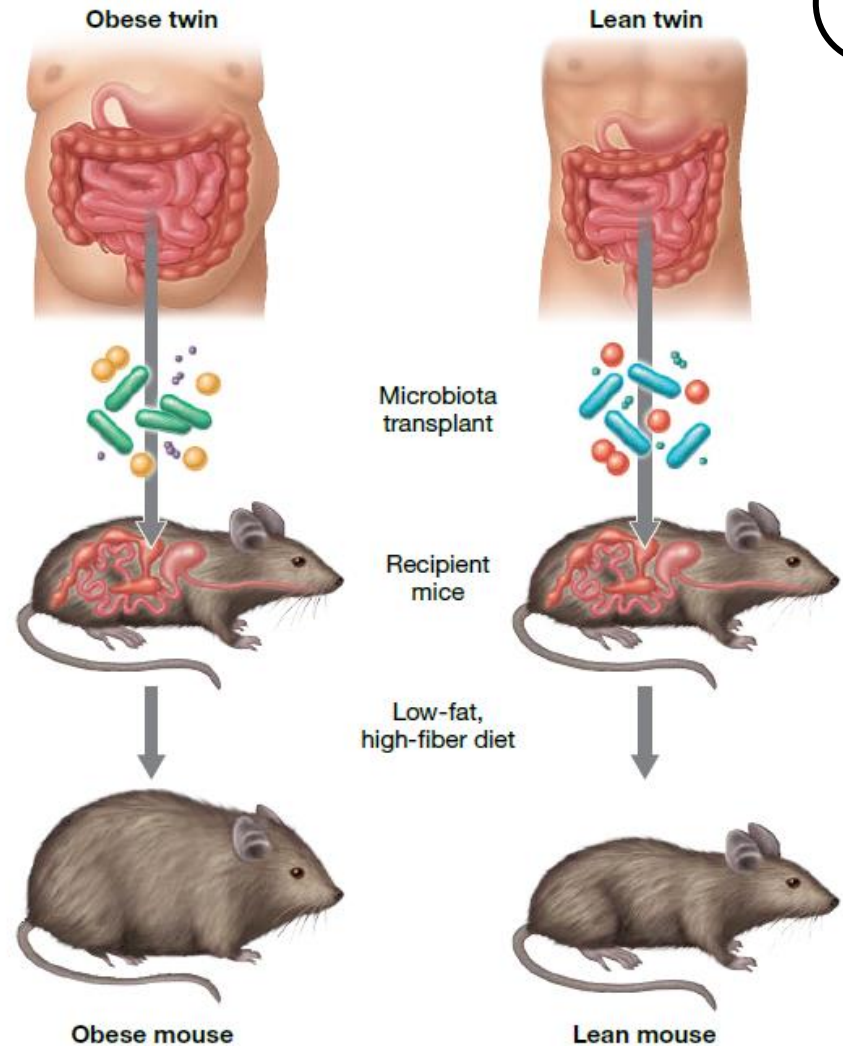


Figure 24.20 Transfer of an obese condition by fecal transplant. Transplanting fecal material from the gut contents of a paired identical human twin study group (one twin was obese and the other lean) to germ-free mice showed that the obese twin microbiota made the mouse obese. Conversely, transfer of gut contents from the lean twin did not contribute to an obese phenotype. Adapted from Ridaura, V.K., et al. *Science* 341: DOI:10.1126/science.1241214.

How lean mice became obese

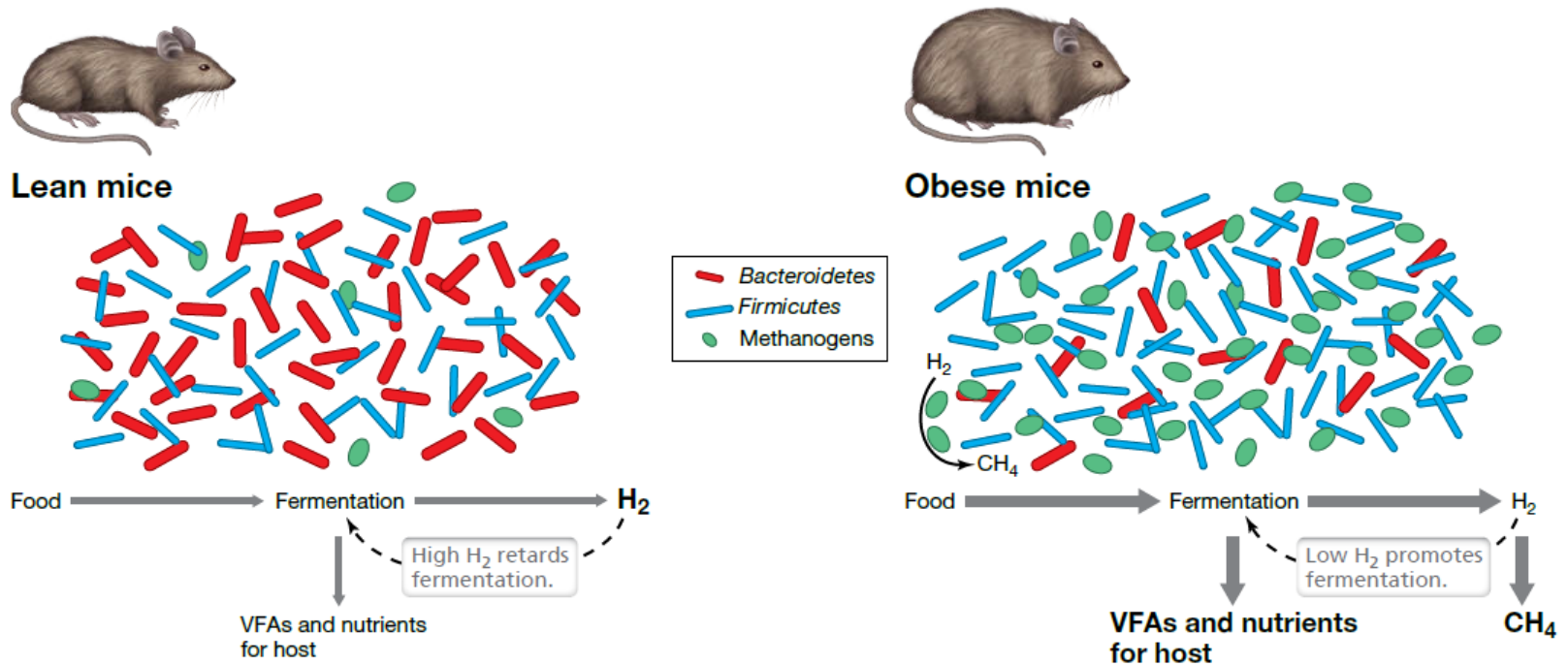


Figure 24.19 Differences in gut microbial communities between lean and obese mice. Obese mice have more methanogens, a 50% reduction in *Bacteroidetes*, and a proportional phylum-wide increase in *Firmicutes*. Nutrient production from fermentation is higher in obese mice due to removal of H₂ by methanogens.

Modulation of the human microbiome

Antibiotics – „kill“ microorganisms which make diseases

Probiotics – “live microorganism which, when administered in adequate amounts, confer a health benefit on the host.”

Mainly *Bifidobacterium* and *Lactobacillus*

Prebiotics – certain plant nutrients that promote microbial growth

GOS – galactooligosaccharides (like HMO)

FOS – fructooligosaccharides (like inulin)

Resistant starches

Synbiotics – combination of both pre- and probiotics

Future seems promising

Biomarkers – predicting predisposition to specific diseases

Microbiota targeted **therapies**

Tailor-made probiotics

Caveat

Cause and effect problem

Microbial studies are still only correlations



Take-home message

1. You are a **superorganism** of human cells and microbes (1.3x more microbial cells, 100x more microbial genes)
2. Main phyla in the gut are *Firmicutes*, *Bacteroides*, *Proteobacteria* and *Actinobacteria*
3. SCFA for gut health – eat plenty of dietary fiber, complex carbs and prebiotic food for gut health
4. *H. pylori* is not always a villain
5. When possible breast feed and deliver vaginally and be careful with ATB prescription

Resources

