Microbes and Men

Martin Krsek

Masaryk University Brno, Faculty of Medicine

Department of Public Health

What is the main role of microbes?!

Degradation

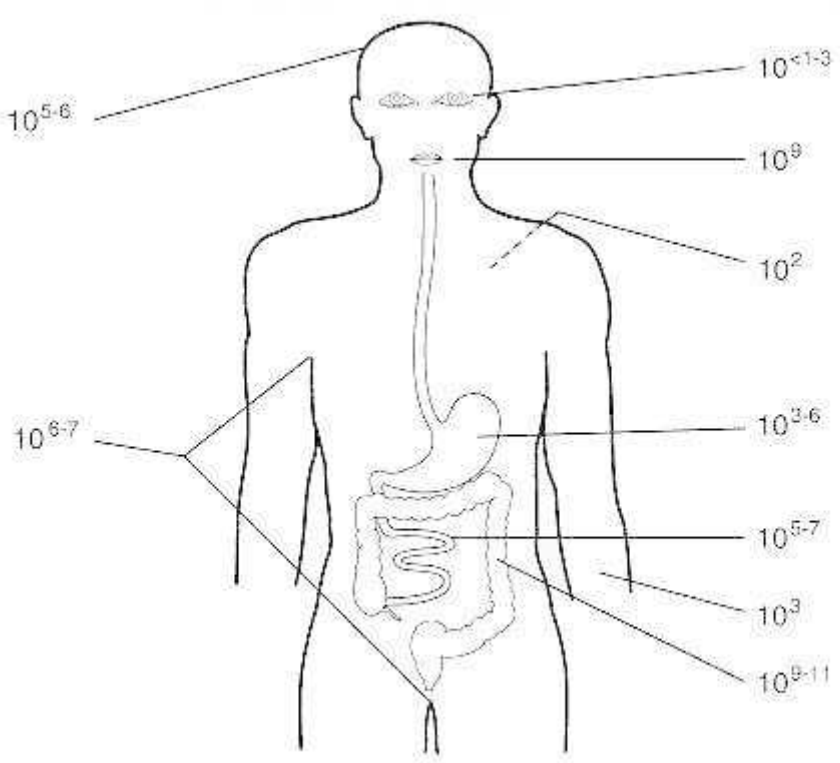
Nutrient cycling

Photosynthesis

Interaction with plants and animals

Keeping live alive/running

How many microbial cells are on/in our body?



Numbers represent the number of organisms per gram of homogenized tissue or fluid or per square centimeter of skin surface.

Skin – 10^{12}, mouth – 10^{10}, intestine - 10^{14}

How many cells are in our body?

How big is a human cell?

cell type	average volume (μm^3)	BNID
sperm cell	30	109891, 109892
red blood cell	100	107600
lymphocyte	130	111439
neutrophil	300	108241
beta cell	1,000	109227
enterocyte	1,400	111216
fibroblast	2,000	108244
HeLa, cervix	3,000	103725, 105879
hair cell (ear)	4,000	108242
osteoblast	4,000	108088
alveolar macrophage	5,000	103566
cardiomyocyte	15,000	108243
megakaryocyte	30,000	110129
fat cell	600,000	107668
oocyte	4,000,000	101664

How many cells are in our body?

5 billion (10^9) – 200 million trillion (10^{18}) ?

Mean weight - 70 trillion cells (10^{12})

Mean volume – 15 trillion cells (10^{12})

Perhaps 37.2 trillion cells (10^{12})

Predominant bacteria at various anatomical location in adults

A diverse **microbial flora** is associated with the skin and mucous membranes of every **human** being from shortly after birth until death. The **human body**, which contains about 10^{13} cells, routinely harbors about 10^{14} **bacteria** . This **bacterial** population constitutes the **normal microbial flora** .

[Normal Flora - Medical Microbiology - NCBI Bookshelf]

https://www.ncbi.nlm.nih.gov/books/NBK7617/

Table 3. Predominant bacteria at various anatomical locations in adults.

Anatomical Location	Predominant bacteria
Skin	staphylococci and corynebacteria
Conjunctiva	sparse, Gram-positive cocci and Gram-negative rods
Oral cavity	
teeth	streptococci, lactobacilli
mucous membranes	streptococci and lactic acid bacteria
Upper respiratory tract	
nares (nasal membranes)	staphylococci and corynebacteria
pharynx (throat)	streptococci, neisseria, Gram-negative rods and cocci
Lower respiratory tract	none
Gastrointestinal tract	
stomach	*Helicobacter pylori* (up to 50%)
small intestine	lactics, enterics, enterococci, bifidobacteria
colon	bacteroides, lactics, enterics, enterococci, clostridia, methanogens
Urogenital tract	
anterior urethra	sparse, staphylococci, corynebacteria, enterics
vagina	lactic acid bacteria during child-bearing years; otherwise mixed

Overall ratio men : microbes 1 : 1-10

Significance of the Normal Flora

The normal flora influences the anatomy, physiology, susceptibility to pathogens, and morbidity of the host.

The normal microbial flora is relatively stable, with specific genera populating various body regions during particular periods in an individual's life.

Three developmental changes in humans: weaning, the eruption of the teeth, and the onset and cessation of ovarian functions

Even though most elements of the normal microbial flora inhabiting the human skin, nails, eyes, oropharynx, genitalia, and gastrointestinal tract are harmless in healthy individuals, these organisms frequently cause disease in compromised hosts.

Significance of the Normal Flora

Germ-free animals (intestinal atonia)

- alimentary lamina propria is underdeveloped, little or no immunoglobulin is present in sera or secretions, intestinal motility is reduced, and the intestinal epithelial cell renewal rate is approximately one-half that of normal animals

Animals treated with streptomycin infected with streptomycin-resistant *Salmonella*.

- 10^6 cells x fewer than 10 to establish a gastrointestinal infection
- fermentation products (acetic and butyric acids) produced by the normal flora inhibited *Salmonella* growth in the gastrointestinal tract.

Normal Flora of Skin

The adult human - 2 square meters of skin- permanent contact with environment

Skin regions = geographic regions of Earth: the desert of the forearm, the cool woods of the scalp, and the tropical forest of the armpit.

- greasy, sweaty (head, neck,…) – production of sebum – *Propionbacterium spp.*
- wet regions – *Corynebacterium spp.*
- dry regions – the highest diversity – *Staphylococcus spp.*

Most bacteria on the skin are sequestered in sweat glands.

Gram-positive cocci: *Staphylococcus epidermidis* – life threatening disease in hospitals - catheters and surgery; and *Micrococcus* sp.;

Normal Flora of Skin

- corynebacteria such as *Propionibacterium* sp. – considered non-pathogenic
- staphylococci and propionibacteria produce fatty acids that inhibit the growth of fungi and yeast on the skin.

but, if *Propionibacterium acnes* - anaerob*,* a normal inhabitant of the skin, becomes trapped in hair follicle, pores and glands, it may cause inflammation and acne
– but at the same time it prevents colonization by more dangerous pathogens.

- different communities even in different layers of skin

- also micromycetes – *Malassezia* spp.
- mainly on legs

Skin microbiota and immunity

P. acnes – strains on healthy skin – genes for **thiopeptides –** inhibition of G+ bacteria (*Staphylococcus epidermis*)
- strains of *P. acnes* responsible for acne do not produce thiopeptides

S. epidermis – secretion of **lipoteichoic acids - ** prevention of release of inflammatory cytokins from skin cells

S.epidermis – on skin of axenic mouse – support of immunity (T-cells)

Various microbes influence different part of immune system.

Some microbes produce antimicrobial substances, other induce release of cytokins connected with illnesses.

Skin probiotics? Return to the original status?

Normal Flora of the Conjunctiva

Historicaly - small numbers of *Staphylococcus epidermidis* and certain coryneforms (*Propionibacterium acnes*)

- blinking wipes the conjunctiva every few seconds
- lachrymal secretions (tears) also contain bactericidal substances including lysozyme

- *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are thought to be able to specifically attach to the conjunctival epithelium

Today – rich microbiota including viruses
Conjunctiva and cornea – about 12 slightly different bacterial genera
1/3 of them – impossible to classify

Normal Flora of the Respiratory Tract

upper respiratory tract (nasopharynx), mainly nares (nostrils) heavily colonized

- *Staphylococcus epidermidis* and corynebacteria
- 20% of the general population - *Staphylococcus aureus* (MRSA)

- healthy sinuses sterile
- pharynx (throat) - streptococci and various Gram-negative cocci
  - sometimes pathogens (*Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae* and *Neisseria meningitidis)*

lower respiratory tract (trachea, bronchi, and pulmonary tissues) nearly free of microorganisms (cleansing action of the ciliated epithelium, coughing, sneezing, swallowing)

- islands of microbes – *Streptococcus spp., Prevotella spp., Veillonella spp*.
- damaged epithelium - *H. influenzae* or *S. pneumoniae* descending from the nasopharynx

Normal Flora of the Urogenital Tract

Urine - sterile, microorganisms have problems gaining access and becoming established.

Anterior urethra - S*taphylococcus epidermidis, Enterococcus faecalis,* alpha-hemolytic streptococci, some enteric bacteria (e.g. *E. coli, Proteus*) and corynebacteria (contaminants)

Vagina

Vagina - various microbiota depending on race , age
- highly individual

- colonized after birth with corynebacteria, staphylococci, streptococci, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*).

During reproductive life the vaginal epithelium contains glycogen. Doderlein's bacillus predominates, being able to metabolize the glycogen to lactic acid. The lactic acid and other products of metabolism inhibit colonization by all except this lactobacillus and a select number of lactic acid bacteria. The resulting low pH of the vaginal epithelium prevents establishment by most other bacteria as well as the potentially-pathogenic yeast, *Candida albicans.* This is a striking example of the protective effect of the normal bacterial flora for their human host.

Vagina

- most of the women - dominate one of the 4 *Lactobacillus* spp. strains
  - sometimes other anaerobic bacteria - but they also produce lactic acid

- dominance of *Lactobacillus* spp.
  80% Asian women and 90% white (pH 4.4 and 4.2)
  60% Hispanic and Black (pH 5.0 and 4.7)

- possible association with premature birth
  on average 10% of premature births
  in an area dominated by black and Hispanic populations it is 20%

- fast changes of microbiota (even 24 hours)

Maternal microbiota

vagina - Lactobacillus
newborn - Actinobacteria, Proteobacteria, Bacteroides
postpartum placenta - *E. coli, Bacteroides, Prevotella tannerae, Neisseria lactamica*
- species composition - the most similar to oral cavity
- metabolic pathways - metabolism of cofactors and vitamins

breast milk - microbial soup
- *Streptococcus* spp., *Staphylococcus* spp., *Serratia* spp., *Corynebacterium* spp., *Lactobacillus* spp., but highly individual
- mother handover microbes from the child's environment - training of immune system

during pregnancy and breastfeeding – changes in maternal microbe – important even for mother‘s health

Maternal microbiota

Mastitis - disappearance of *Lactobacillus spp.*, dominance of one strain of pathogenic bacteria

Adding lactobacilli to food - the same bacterium has been shown in milk after a three-week cure – disappearance of problems - better than antibiotics?

Breast - their own microbiota even at non breast feeding woman - meaning for cancer?

Normal Flora of the Oral Cavity

1676 - Anthony van Leewenhoek - "animacules"
1890 - W.D. Miller - The Microorganisms of the Human Mouse
2016 - Jakubovics - structure of dental plaque

500-600 (700) of different bacterial species (30% non-cultivated)
streptococci, lactobacilli, staphylococci, corynebacteria and anaerobic bacteria (bacteroides)

a common development with us for millions of years
- use of fire, agriculture, processed food (sugar),
- antimicrobial therapy

age-dependent:
- after birth - *Streptococcus salivarius* (98%)
- tooth eruption - *S. mutans* (lactic acid) and *S. sanguis*
- around puberty - Bacteroides and Spirochetes

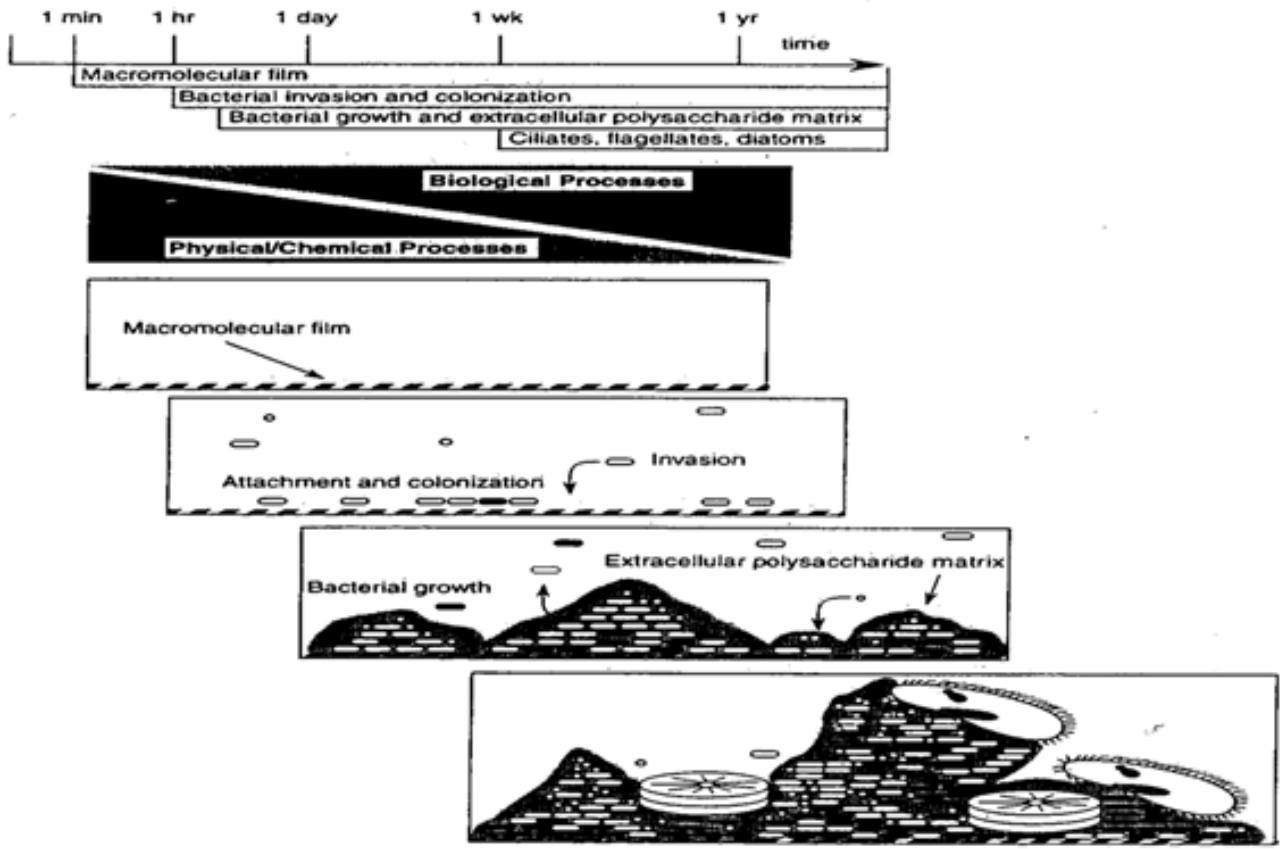
Normal Flora of the Oral Cavity

Three Habitats
- mucous membranes of the cheeks, gums, hard palate - Firmicutes, *Streptococcus* sp.
- throat, tonsils, back of the tongue, saliva – *Veillonella* spp., *Neisseria* spp.and *Leptotrichia* spp.
- dental plaque – *Capnocytophaga* spp., *Actinomyces* spp., *Rothia* spp. and *Corynebacterium* spp.

Mouth - warm, moisture, nutrients - saliva, crevicular fluid of gums
- proteins, glycoproteins
- immunoglobulin A, lactoferrin, lactoperoxidase, lysozyme, staterin, histatin

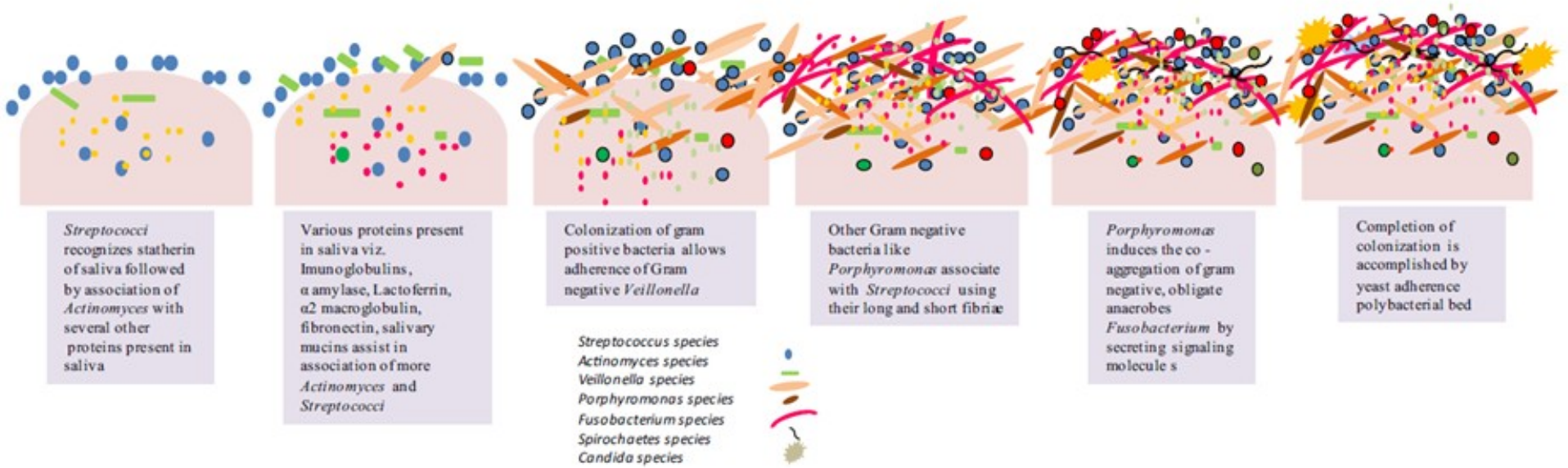
- result is a balanced microbiota
- these substances together with other (even microbial) form a biofilm allowing microbes to hold and at the same time protect teeth against acid attack

Biofilm in general



The formation of a biofilm occurs as a successional process. The first step is the physical conditioning of the surface with the deposition of substances that attract bacteria and permit their adherence and growth. Next, populations invade, attaching to the surface and colonizing it. These bacteria initially form a monolayer. As they reproduce to form a thicker layer, new bacteria invade to establish a community with multiple populations. These bacterial populations excrete extracellular polysaccharides that form a matrix within which the biofilm's bacterial populations adhere. Eucaryotic microorganisms, including algae and protozoa, then invade the biofilm, continuing the successional process to form a complex biofilm community that is highly resistant to outside disturbance. (Source: Lawrence et al. 1995)

Normal Flora of the Oral Cavity



- at first, the physical process - covering the surfaces with a film of dissolved substances
  - then (minutes) - reversible attachment - irreversible
  - oral bacteria - various adhesions - interactions with molecules and receptors on other bacteria
  - *Streptococcus* spp., *Actinomycetes* spp., *Veillonella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Spirochetes* spp., *Candida* spp.

Normal Flora of the Oral Cavity

Biofilm

- the coadhesion of late colonizers to previously attached ones
- increased diversity and biofilm biomass
- the importance of bacterial polymers (glucans, fructans, heteropolymers)
- matrix - nutrients, water, enzymes
  - gradient - pH, O_2, nutrients
- regulation of gene expression - different from the same planktonic microbes
- facilitated horizontal gene transfer
- quorum sensing peptides increased the frequency of transformation in *S. mutans* 10-600x
- increased resistance to antimicrobial agents

Normal Flora of the Oral Cavity

Viruses
- pathogenic - mumps, rabies, hepatitis, HIV, respiratory infections
- most but bacteriophages - lytic, lysogenic - .....

Fungi
- 85 genera were detected
- *Candida spp., Cladosporium spp., Aureobasidium spp., Saccharomycetales spp., Aspergillus spp., Fusarium spp.,* and *Cryptococcus spp.*
- *C. albicans* - its presence allows the growth of anaerobes in aerobic conditions

Archea
- minor component
- metanogens - especially people with periodontitis

Normal Flora of the Oral Cavity

Bacteria

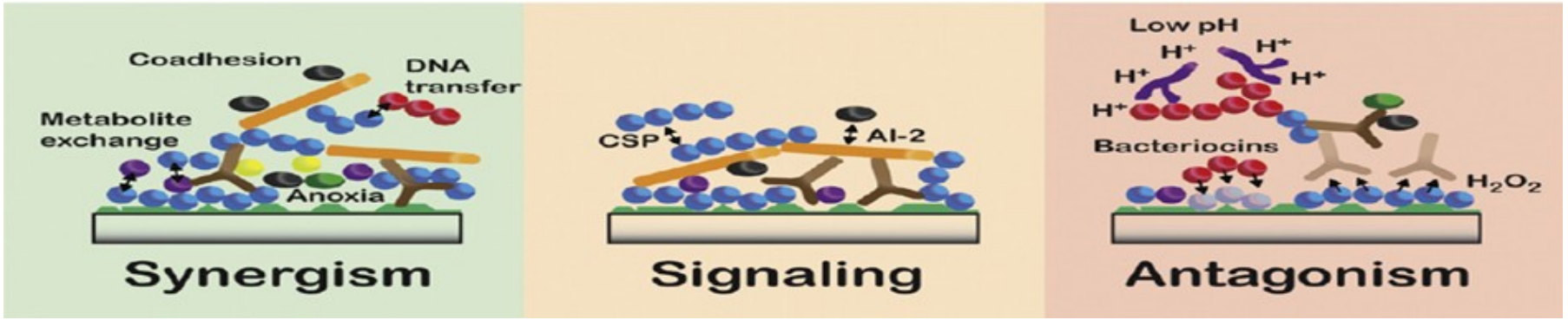
Expanded Human Oral Microbiome Database (eHOMD)
- November 22, 2017 - 772 prokaryotic species (70% cultivated)
- 16S rDNA profiling - 6 groups: Firmicutes, Actinobacteria, Proteobacteria, Fusobacteria, Bacteroids and Spirochaetes
  = 96%

- the discovery of ultra-small bacteria (not just in the oral cavity)
  - reduced genome, absence of many biosynthetic and metabolic pathways including the electron transport chain
  - obligatory symbionts?

Normal Flora of the Oral Cavity

Dental plaque

- bacteria living side by side use different nutrients, produce different substances:
    - synergism, induction of response in target cells and competition



- the same substance - different functions – according to situation, concentration
- H_2O_2 - inhibition, but in sub-lethal concentrations signaling function
- likewise organic acids, bacteriocins - antagonism, synergism
- bacteriocins – induction of cell competence, DNA release

The role of normal oral cavity microflora

- the mere presence inhibits colonization of GIT by potential pathogens

- **nitrate metabolism** and cardiovascular disease
  - ¼ of the received nitrate returns to the oral cavity (saliva ...)
  - the microbiota converts nitrates into nitrites - into the blood and transformed into NO
  - NO - vital to vascular health - flexibility, elasticity – blood pressure reduction
  - but also erections, relaxation of the muscles in the digestive system - transport of food
  - the use of antibacterial mouthwash significantly reduced the intake of nitrite, eliminating the effect of nitrite on decrease of blood pressure

  - also connection with other systemic diseases:
  - metastatic infections of heart, brain, spleen, pancreas, liver, and bone
- - arthrosclerosis, cardiovascular diseases, stroke,
  - respiratory diseases, meningitis, pneumonia, diabetes

The role of normal oral cavity microflora

Negative effects

- dental caries, gingivitis, periodontitis
  - UK - 46% of 15-year-old children - decay in permanent teeth
  - 45% of adults - perionditis

Tooth decay

- dissolving the teeth structure by acid from sugars, reducing buffering capacity of saliva and thus reducing pH in mouth
- change of oral microbiota in the benefit of acidophilic species - *Streptococcus mutans* and lactobacilli - further acid production

S. mutans is not the only one – *Bifidobacterium spp., Propionibacterium spp., Scardovia spp.*
Some bacteria, on the contrary, increase the pH (urea, arginine - ammonia)

- the same obligatory anaerobes are enriched in both inflammatory and tumorous tissues

The role of normal oral cavity microflora

gingivitis

- 90% of adults
  - dental plaque - primary colonizers - G + aerobic and fac. anaerobic bacteria (streptococci and Actinomyces)
  - mature plaque - G- anaerobic – *Fusobacterium spp., Treponema spp., Synergistetes spp.*
  - regular teeth brushing- plaque "does not mature"
  - neglected hygiene - endotoxins and other enzymes into the gums – irritation and inflammation
  - loss of gum and tooth connections - periodontal pocket
  - anaerobic colonization - host reaction (protease formation) makes the situation even worse
  - teeth release
- *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Anaeroblobus geminatus, Eubacterium saphenum, Allocus filipactor, Porphyromonas endodontalis, Prevotella denticola* and others

The role of normal oral cavity microflora

Healthy mouth - most bacteria in symbiosis with the host
Disease - increased occurrence of cariogenic and periodontopathic bacteria
- disturbance of balance - dysbiotic status

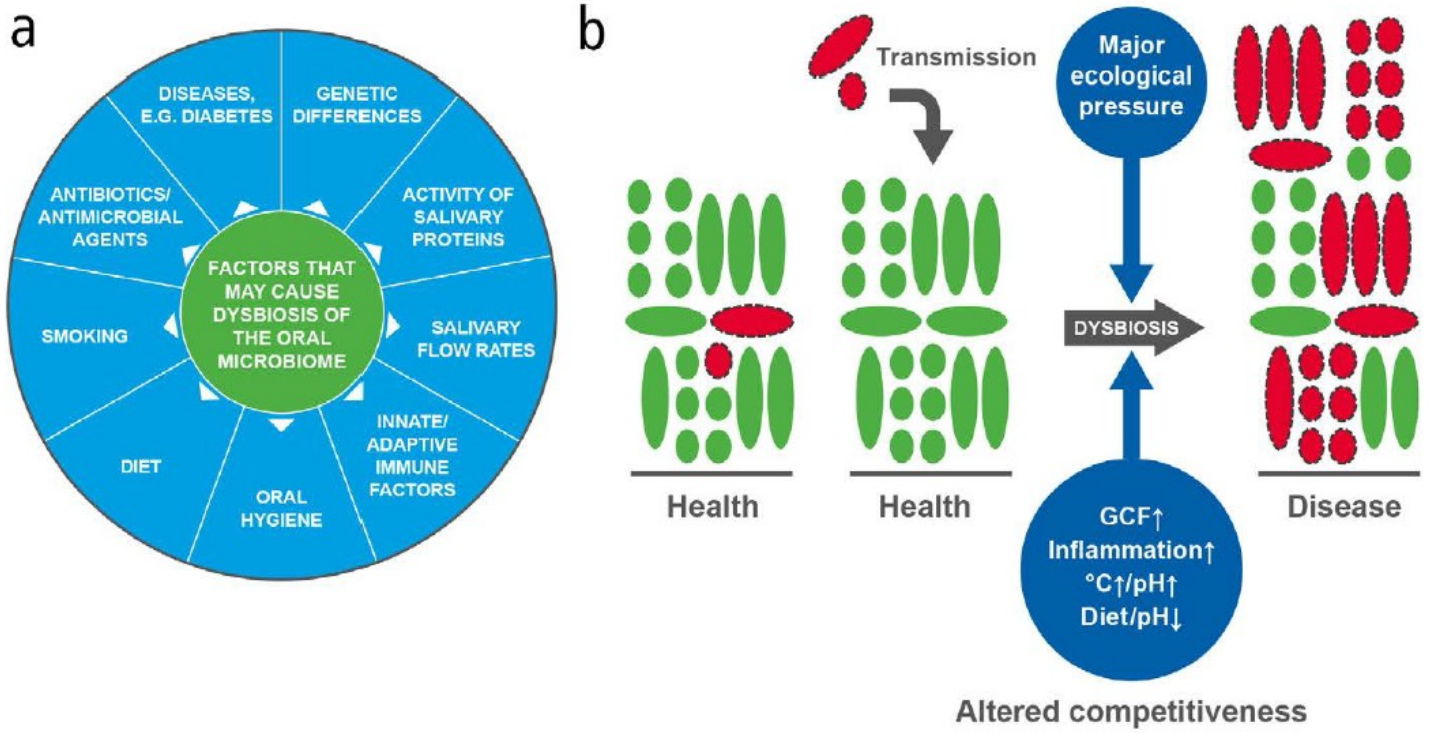


Fig. 5 (a) Causes of dysbiosis; (b) A model of dysbiosis (adapted from Marsh[80])

Dental plaque and dental diseases

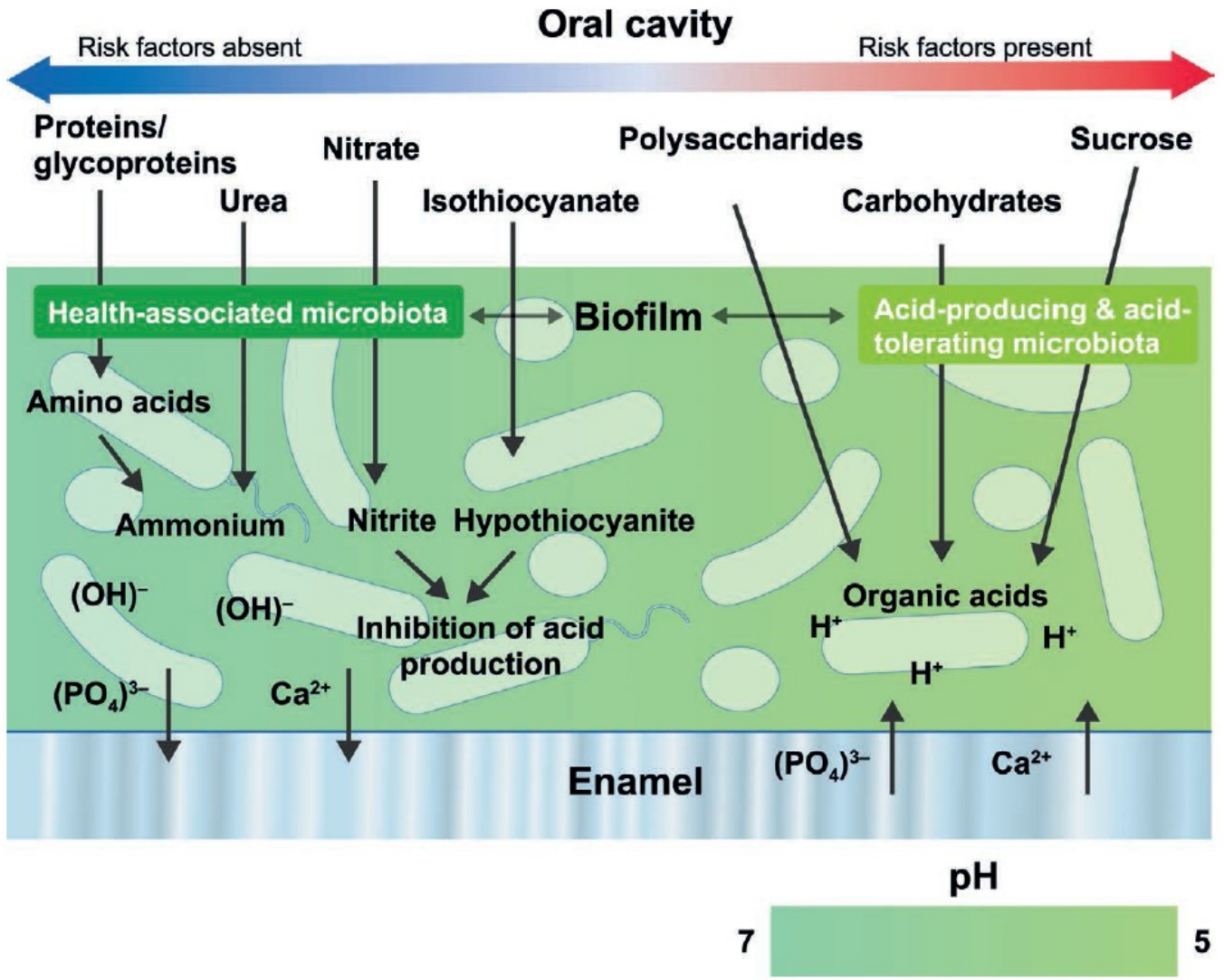


Fig. 6 A contemporary model of host–microbe interactions in the pathogenesis of caries (adapted from de Soet & Zaura and Takahashi)[97,51]

Dental plaque and dental diseases

Non-specific Plaque Hypothesis (19th Century)
- dental infections caused by non-specific increase of all bacteria - remove the maximum

Specific plaque hypothesis
- diseases caused by a few species, their removal will solve the problem

Ecological Plaque Hypothesis (1980)
- significant changes in the plaque environment change relationships between bacteria
- enrichment of some species.
- disease prevention not only by inhibiting pathogens but also by changing conditions of the environment.

Normal Flora of the Oral Cavity

Dentures
- more aerobes, yeast and lactobacilli
- on denture - *Candida albicans*
- under denture - a more acidic environment, fewer saliva - again *C. albicans*

Tooth decay
- vaccination, genetically modified *S. mutans*
- BUT – *S. mutants* can be replaced by other bacteria - *S. pneumoniae*

- **benefits of microbes** - vitamins, inducing low levels of circulating and secretory antibodies, antagonism against nonindigenous species (fatty acids, peroxides and bacteriocins)
- **at the same time** – diseases: abscesses, dental caries, gingivitis, periodontal disease but also abscesses of alveolar bone, lung, brain, or the extremities (*Bacteroides melaninogenicus*)

Microflora of the oral cavity and tooth brushing

- cleaning teeth for several thousand years before Christ
  - but regular use of brushes and pastes only after World War II

  - various teeth cleaning techniques
  - manual x electric brush
  - choice of paste
- - ads - sterile oral cavity ?!

Normal Flora of the Gastrointestinal Tract

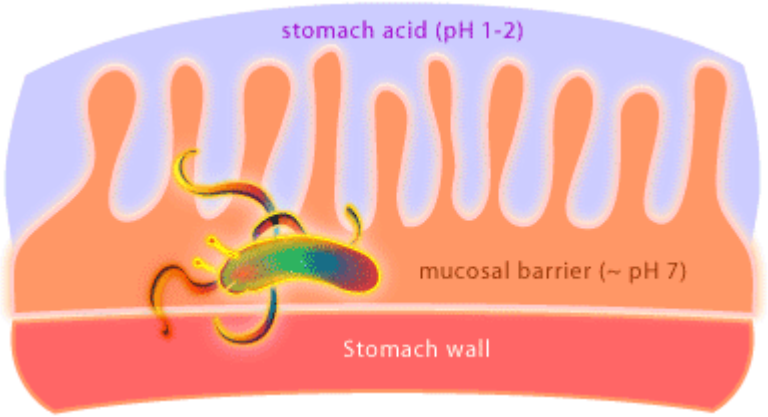
Birth - bacteria enter with the first food
- **breastfed babies** - bifidobacteria (90%), *Enterobacteriaceae* and enterococci
- **artificial nutrition** - bifidobacteria do not dominate
- switching to **cow's milk** or solid food - bifidobacteria plus enteric bacteria, bacteroides, enterococci, lactobacilli and clostridia
- **human milk** - a growth factor supporting the growth of bifidobacteria
- bifidobacteria prevent colonization by non-indigenous or pathogenic species
- evidence of "tissue tropism" and specific adherence

- **gram-positive bacteria** - gastrointestinal epithelium – polysaccharides of capsules or teichoic acids of the cell wall are bound to specific epithelial cell receptors

- **gram-negative bacteria** - specific fimbria binds to glycoproteins on epithelial cell surface

Normal Flora of the Gastrointestinal Tract

- age, diet, cultural conditions, use of antibiotics, …
- esophagus – none bacteria
- stomach – pH - acid-tolerant lactobacilli
    - *Helicobacter pylori* - gastric ulcers, gastric and duodenal cancer
    - urease – urea – ammonium
    - maybe also positive functions - protection against infant diarrhea and esophageal disease.



Normal Flora of the Gastrointestinal Tract – cont.

- **small intestine -** high flow rates - lactobacilli and *Enterococcus faecalis* - 10^5 - 10^7
- distal part (10^8/ml) - additional species - *E. coli* and relatives and *Bacteroides*
- digestion mainly by human enzymes, microbes could compete for essential nutrients

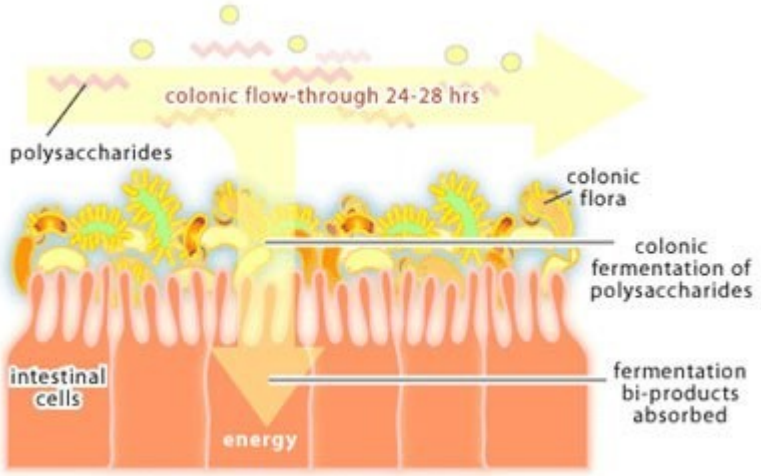
- **large intestine** (colon) - similar microbes as in feces - 10^{11}/ml or even more – 10^{13}
- bacteria - about 35-50% of the colon contents - 2 lbs
- coliforms, enterococci, clostridia and lactobacilli
- predominant anaerobic *Bacteroides* and anaerobic lactic acid bacteria *Bifidobacterium* (*Bifidobacterium bifidum*)
- significant numbers of anaerobic methanogens

Normal Flora of the Gastrointestinal Tract – cont.

Role of bacteria in GI tract

- protection from infection by alien microbes
- contribution to nutrition and digestion (polysaccharides – acetate, butyrate and propionate - source of carbon and energy for mucosal cells of the colon)
- production of vitamins
- stimulation of development and activity of the immunological tissues

- **BUT ALSO:**
- production of carcinogenic metabolites (colon cancer)
- alterations in the GI flora (poor nutrition, antibiotics)
  - shifts in populations and colonization by nonresidents
  - gastrointestinal disease



Viruses and our microbiota

- majority of known viruses are pathogenic - because we learned just about them (diseases)
  - but many viruses infect us without any disease symptoms
  - molecular methods - many other viruses discovered
  - originally 2 human polyomaviruses - today 13 - infect us during childhood and then waiting

- Koch's postulates - a problem with viruses (cultivation in pure culture)

- majority of viruses are neither completely pathogenic nor harmless - according to the situation

Viruses and our microbiota

Benefits of viruses

- childhood infections stimulate the development of the immune system - protection against later infection and exaggerated reaction – allergies

- comensal virus may provide protection against a pathogenic virus
- pegivirus C (related to hepatitis C, Zika virus, dengue fever) mitigates the effects of HIV
  - ¾ billion people are infected
- viruses prefer fast dividing cells - cancerous - spontaneous regression

- about 8% of our genome are retroviral DNA sequences
- some of their functions are essential for host survival and development

Mycobiota

- 2015 - only 269 out of 6,000 results for microbes mentioned fungi
- mykobiome - only 55 results

- oral mycobiota of HIV patients (*C. albicans*)

- more severe symptoms of hepatitis B correlate with a number of species of *Candida* spp. and *Saccharomyces spp.*

- the excessive presence of *Candida tropicalis* exacerbates the symptoms of inflammatory bowel disease (IBD)

- undisturbed microbial community - fungi harmless or even beneficial
- after disruption - possible problems

Mycobiota

- oral cavity - not just *Candida* spp. and *Saccharomyces* spp.
  - study on 20 volunteers - 101 fungi identified, each with 9-23 species
  - *Cladosporium* spp. (asthma), *Aureobasidium* spp.(transplant), *Aspergillus* spp. (devastating infections), *Fusarium* spp. (difficult infections), *Cryptococcus* spp. (meningitis in HIV)

  - elsewhere on the body of *Malassezia* spp. - skin diseases
  - lungs - *Aspergillus* spp. in healthy people, *Candida* spp. in patients (cystic fibrosis, cardiovascular diseases, ...)

- inflammatory bowel disease - other mycobiota than in healthy people
- obesity - more *Ascomycota* spp. representatives, but overall reduced diversity
- *Mucor* spp. - slim people (correlation with obesity)

Do we all have the same microbes?

Differences: digestive tract, vagina (species and metabolic diversity)

Similarities: microflora of the mouth and skin - changes in time, but less variable in different individuals

Establishment of human microbiota

- **fetus** in the womb is not sterile
- **vaginal** microflora varies during pregnancy (80% *Lactobacillus* sp.)
- **newborn** - Actinobacteria, Proteobacteria, Bacteroides
- microbiota of the **placenta** - a non-pathogenic comensal microbiota:
  Firmicutes, Tenericutes, Proteobacteria, Bacteroides and Fusobacteria
  (*E. coli, Prevotella tannerae, Neisseria lactamica*) - similar to the oral microflora
- further colonization during **delivery**, then **breastfeeding** (milk is not sterile),
  from the mother's skin
- **milk**: *Streptococcus spp., Staphylococcus spp., Serratia spp., Corynebacterium spp.*
  depends on mother's environment (preparation ...)
- also baby is exploring the environment by mouth
- around the second year of life a certain balance is achieved

Manipulation of human microbiota

- 4th century **Chinese** medical literature mentions faecal transplant to treat food poisoning and severe diarrhea

- Bedouins – camel faeces for bacterial dysentery

- example of successful manipulation of our microbiota is faecal transplant (stool bank - OpenBiome) for treatment of infections caused by *Clostridium difficile*

But first WE MUST KNOW our microbiota, only then we can think about targeted manipulation

Clostridium difficile

C.difficile
- anaerobic gram-positive spore-forming bacteria
- transmitted by fecal-oral route
- colonizes the large intestine

- release of exotoxins (TcdA, TcdB) causes colitis
- typically after recent antibiotic exposure (penicillins, cephalosporins, clindamycin, fluoroquinolones)

Presentation:

Abdominal pain
Fevers
Diarrhea, can be bloody
Dehydration

Clostridium difficile – cont.

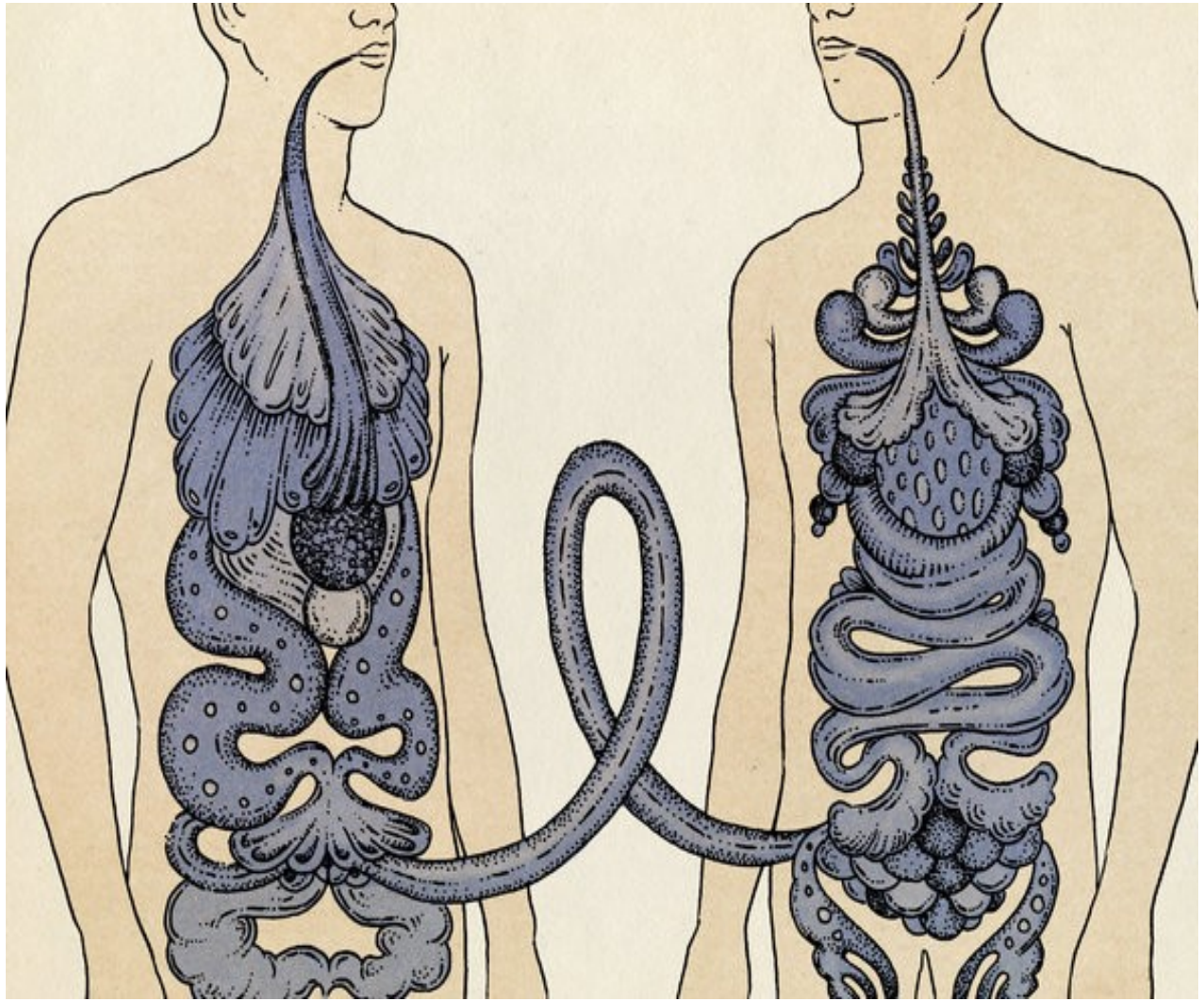
Diagnosis:

- Stool testing for toxin
- Sigmoidoscopy

Oral **antibiotics** to treat CDI

- Metronidazole, Vancomycin → ~70-75% effective
- Fidaxomicin for Vancomycin failure → 90% effective

Clostridium difficile – cont.



Fecal transplant

Donor contraindication:

Donors should not:

- Have had any antibiotic exposure in the past six months
- Be immunocompromised
- Have had any tattooing or body piercing in past six months
- Have any history of incarceration
- Have recently traveled to endemic areas
- Have any chronic GI disorders, such as inflammatory bowel disease

Faecal transplant cont.

Donor screened for:

hepatitis A, B, C

HIV

syphilis

C.difficile

Giardia,

E.coli, Salmonella, Shigella, H.pylori, Campylobacter infections

Faecal transplant cont.

Recipient

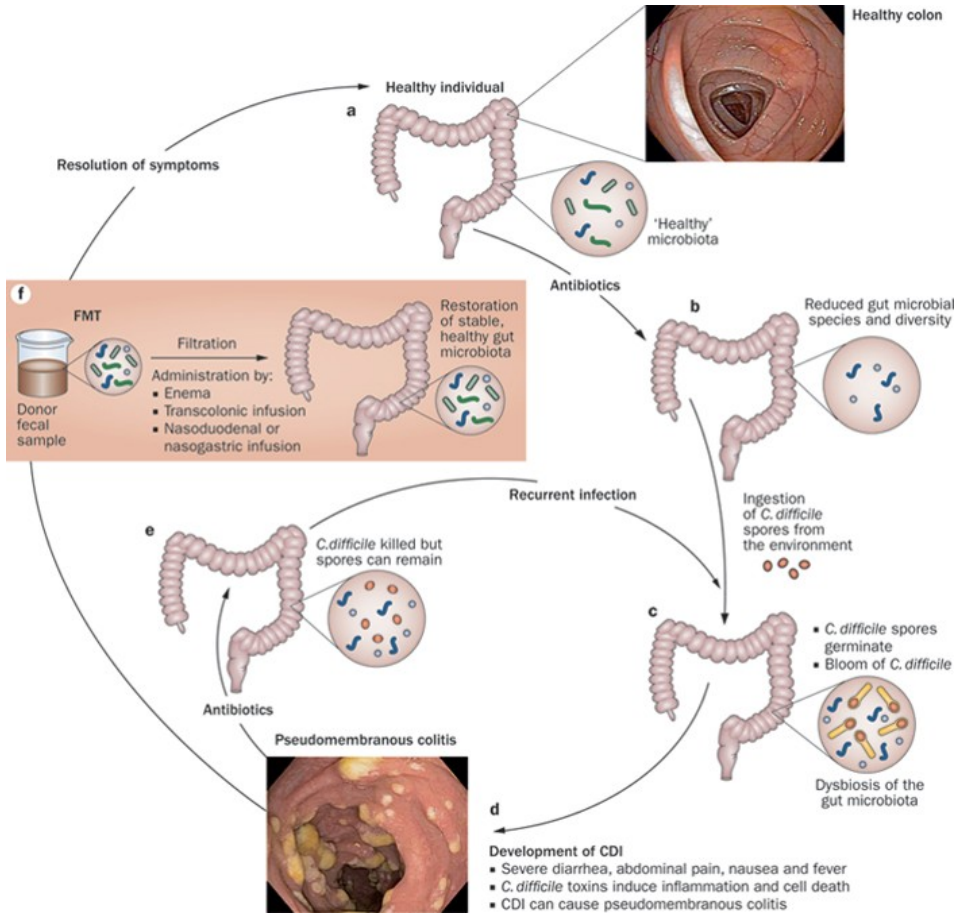
Before procedure:

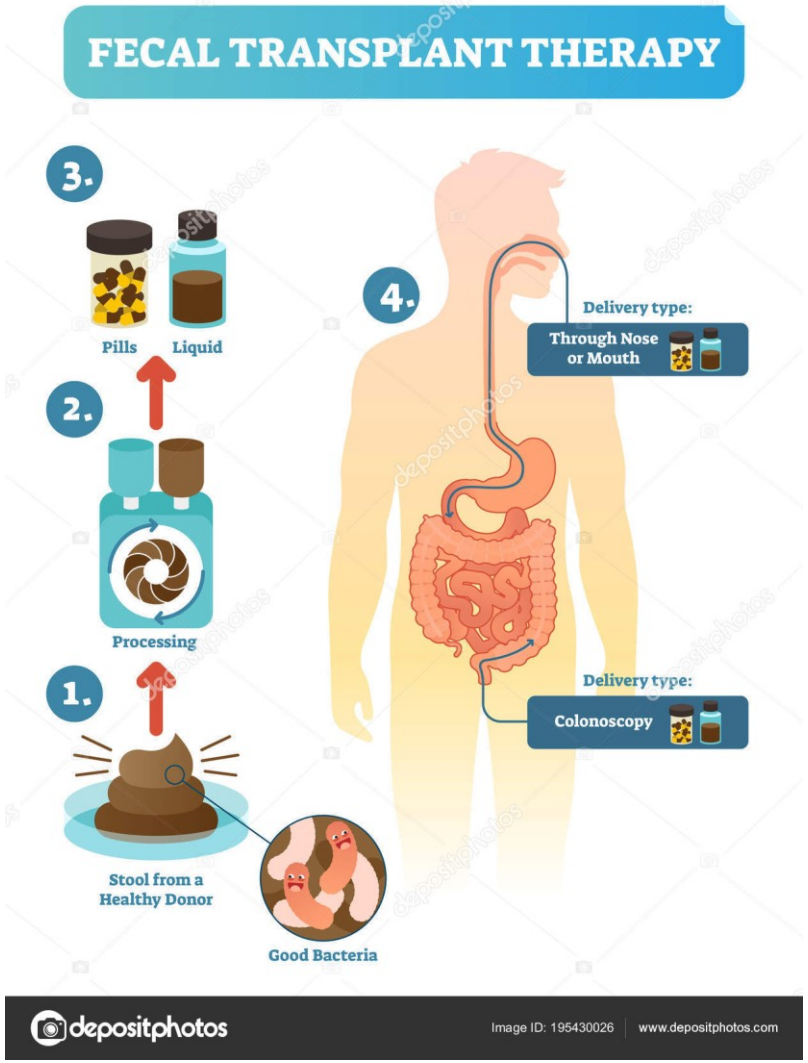
- stop any antibiotic therapy two days before the procedure
- a liquid diet followed by laxative preparation the night before scheduled procedure

Faecal transplant cont.

- Fresh stool collection
- less than 6 hours prior to procedure
- volume 20 ml to 30ml mix 250 ml of sterile 0.9% normal saline with stool in a blender

Faecal transplant cont.





Faecal transplant cont.

Risk of aspiration if not delivered deep into upper small intestine

Risk of acquiring infection from donors – rare

Risk of complications from sedation and endoscopy – bleeding, perforation, transmission of other infections: 1/1000-1/10,000

Manipulation of human microbiota

- **Probiotics** – 100 years history – Mutaflor

- **MUTAFLOR® IS A PROBIOTIC THAT COMPRISES AS ITS ACTIVE SUBSTANCE A VIABLE NON-PATHOGENIC STRAIN OF ESCHERICHIA COLI (E.COLI)**
- **FIRST ISOLATED IN 1917 BY PROFESSOR ALFRED NISSLE FROM THE FAECES OF A SOLDIER DURING THE FIRST WORLD WAR WHO AS HE REMARKED, "IN CONTRAST TO THE LARGE MAJORITY OF HIS COMRADES, HAD SUFFERED NEITHER FROM DYSENTERY NOR FROM ANY OTHER INTESTINAL DISEASES". THEREAFTER, APPROPRIATELY NAMED *ESCHERICHIA COLI* STRAIN NISSLE 1917.**

- **USED TO** relief and management of chronic constipation

Characteristics of Escherichia coli strain Nissle 1917
- the ability to colonize
- antagonistic activity – Inhibition of growth and or killing of pathogens
- anti-invasive – prevention of colonisation in the gut by pathogens
- synthesis of endogenous antimicrobial peptides-defensins
- mucosal integrity – contributing to luminal metabolism and stability of intestinal milieu-enhanced epithelial barrier function
- anti-inflammatory and immunomodulatory effects
- stimulation of colonic mucosa

- Adminitered to **Hitler** by his doctor Theodor Morell during second world war

Manipulation of human microbiota

- **prebiotics** – non-digestible part of our food
  - cellulose, hemicellulose, lignin
  - but also polyphenols (resveratrol – red wine), minerals
  - vitamins - riboflavin
- lactating mothers produce oligosaccharides that are not digested
  - bait for pathogens?
  - supporting beneficial bacteria (able to digest them)?

- microbiome influences/modifies efficiency administered medications

Prebiotika – pokr.

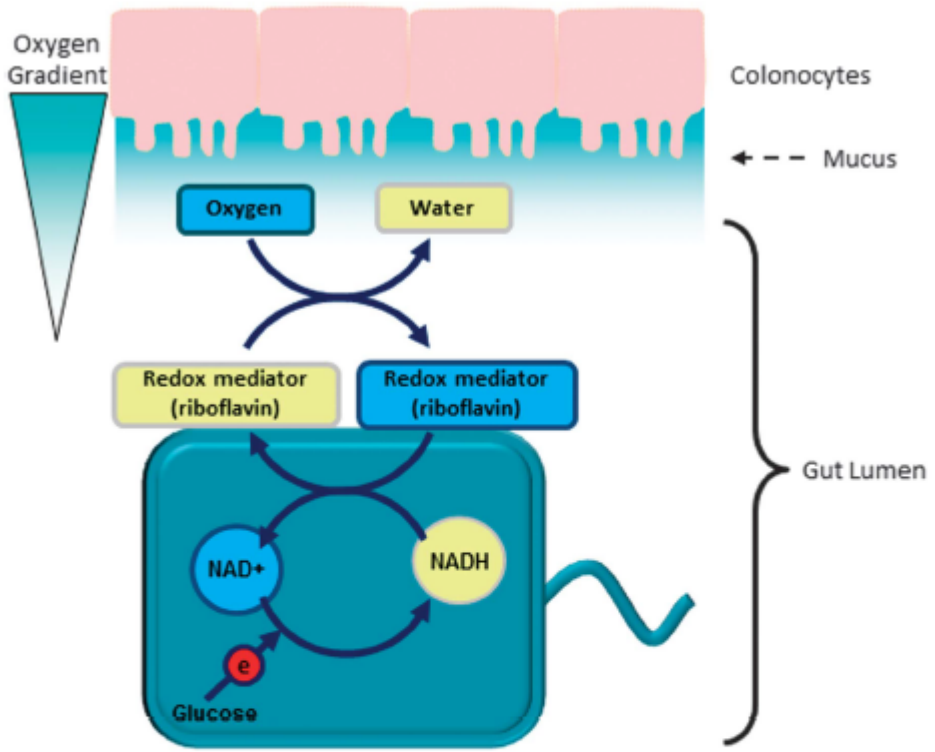


Figure 1. Schematic representation of the function of vitamins as redox mediators carrying reduction equivalents that are liberated during the glycolysis of carbohydrates via NADH to oxygen. Glucose is an example of a carbohydrate electron donor. The electrons reduce the mediators and finally oxidize electron acceptors from the epithelium, such as oxygen or nitrate. The flagellated box represents a bacterium, such as *F. prausnitzii*. In the presence of redox mediators, the anaerobic bacteria can reduce the oxygenated environment using their metabolism and thus reduce oxidative stress.[69,70]

Steinert 2016

Experiments with riboflavin:

- Riboflavin increased the incidence of *Faecalibacterium prausnitzii*
- this bacterium is responsible for production of anti-inflammatory peptides
- for example IBD (inflammatory bowel disease) is characterized by low numbers of *F. prausnitzii*

Table 2. Companies developing microbiome therapies*.

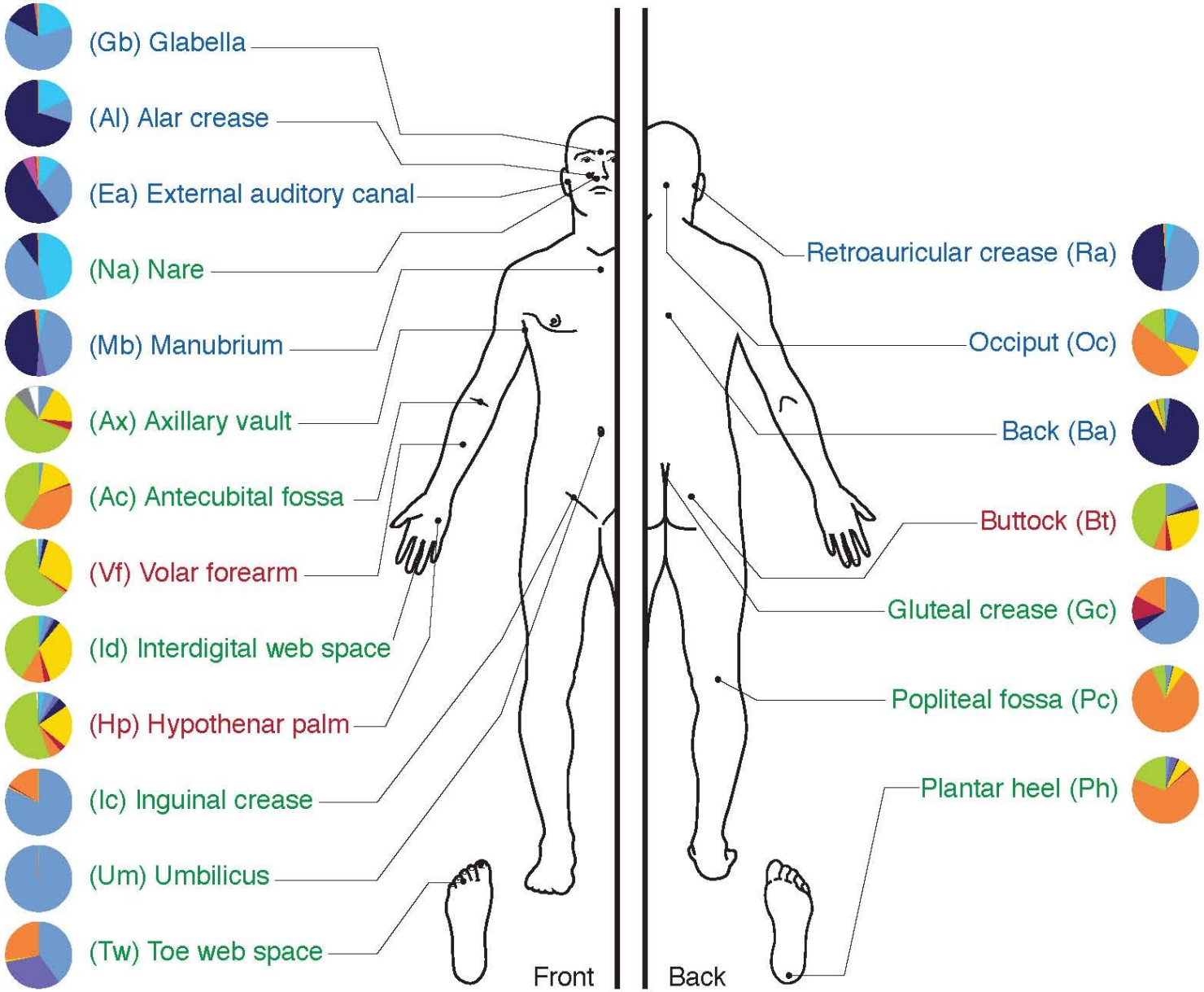
Company	Programs and Status
Rebiotix [111]	Developing a new kind of biological drug designed to reverse pathogenic processes responsible for disease through the transplantation of live human-derived microbes into a sick person's intestinal tract. Rebiotix focus is new solutions for challenging gastrointestinal diseases. The company has completed the Phase 2 open-label clinical trial to assess the safety of RBX2660 (microbiota suspension) for the treatment of recurrent *Clostridium difficile* infection
Enterome [119]	Development of novel drugs and diagnostics to support personalized therapies in microbiome-related diseases such as Inflammatory Bowel Diseases (IBD), metabolic diseases, and related disorders. Three programs at the validation phase: IBD 120 Ulcerative colitis-relapse prediction; MET 220 Bariatric surgery-outcome prediction; MET 230 Stratified nutrition test. One program at discovery phase: IBD 110 Crohn's disease-activity monitoring
Second Genome [120]	Development of microbiome modulators, which are bioactive therapeutics that benefit human health by altering the composition and activities of the microbial communities in the body to regulate host pathways. Second Genome's focus is on microbiome modulators (small molecules, peptides and bacteria) that impact infection, immunity and metabolic disease. The company is currently pursuing three preclinical programs and additional discovery efforts
4D Pharma [121]	Developing biotherapeutics using live bacteria, as opposed to traditional drugs based on chemically synthesized small molecules and antibodies. 4D Pharma has two ongoing programs. Thetanix: for treatment of paediatric Crohn's disease. Blautix: for treatment of Irritable Bowel Syndrome (IBS)
Seres Health [122]	Developing Ecobiotic® therapeutics (combinations of a small number of selected discrete organisms) to treat a range of important medical conditions based on the microbiome biology at their core. SER-109 therapeutic for recurrent *C. difficile* is in Phase 3 trials. Pre-clinical pipeline includes SER-262 for primary *C. difficile* and SER-155 for drug-resistant bacteria. Therapeutics for inflammatory and metabolic diseases are in discovery phase
Microbiome therapeutics [123]	Development of microbiome modulators- products designed to alter bacterial populations and their environment in the gastrointestinal tract to prevent and treat serious health conditions. The company initial research and products are focused on metabolic conditions including prediabetes, diabetes and obesity. The lead microbiome modulator, NM505 is in clinical development to assess its efficacy and safety as a reformation of metformin, the most widely prescribed drug for the treatment of type 2 diabetes. NM504 is in clinical development as a prescription for treatment of prediabetes and diabetes

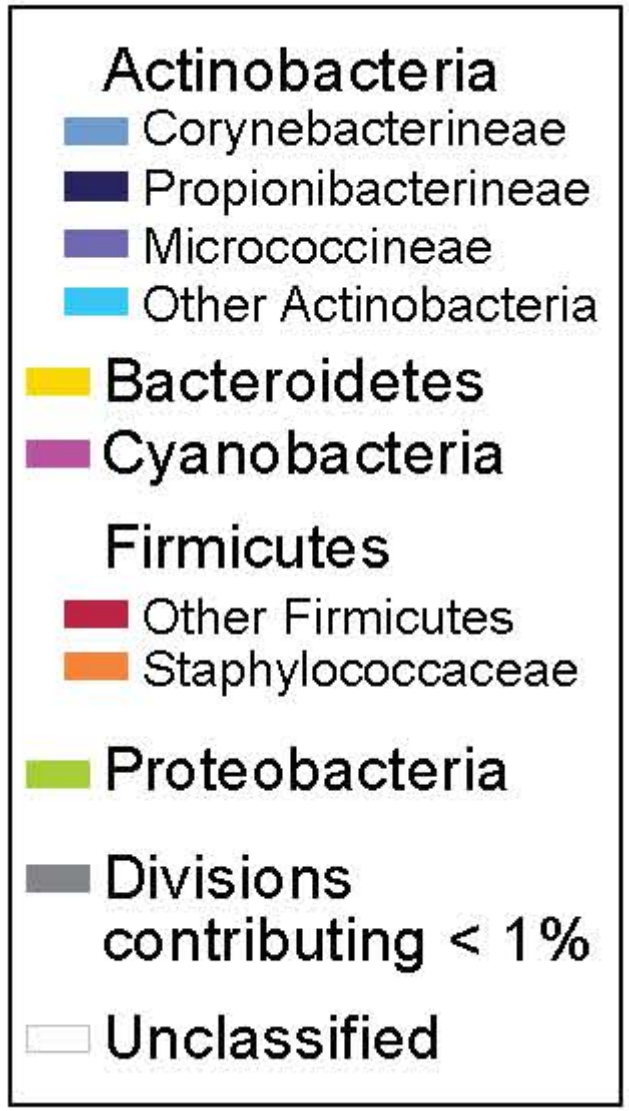
Human Microbiome Project

- launched in 2008 - five-year project - total budget of $115 million
- **(Human Genome Project -** launched in 1990 and was declared complete in 2003)
- oral, skin, vaginal, gut, and nasal/lung – from 300 healthy subjects
- how changes in the human microbiome are associated with human health or disease
- **BUT** – what was here first ?!?!?!?!
- many more (specialized) studies of human microbiome are underway:
  - American Gut Project, MetaHIT, MyMicrobes, Human Longevity, Earth Microbiome project, Human Food Project

RESULTS:

- dramatic variation between individuals as far as species composition is concerned
- **BUT** when classified by function - very similar
- each individual´s microbiome is fairly stable over time
- main problem of interpreting results – public databases


(Gb) Glabella
(Al) Alar crease
(Ea) External auditory canal
(Na) Nare
(Mb) Manubrium
(Ax) Axillary vault
(Ac) Antecubital fossa
(Vf) Volar forearm
(Id) Interdigital web space
(Hp) Hypothenar palm
(Ic) Inguinal crease
(Um) Umbilicus
(Tw) Toe web space
Retroauricular crease (Ra)
Occiput (Oc)
Back (Ba)
Buttock (Bt)
Gluteal crease (Gc)
Popliteal fossa (Pc)
Plantar heel (Ph)
Front
Back


Actinobacteria
Corynebacterineae
Propionibacterineae
Micrococcineae
Other Actinobacteria
Bacteroidetes
Cyanobacteria
Firmicutes
Other Firmicutes
Staphylococcaceae
Proteobacteria
Divisions contributing < 1%
Unclassified

Importance of human microbiota

- up to 2015 – about 19 000 papers published (5,000 in 2015 alone)
- majority is descriptive – identification of which microbes are there
- but what they are doing there?
- how they cooperate in between them and with human host?
- human microbiome includes also viruses, archaea, fungi, single-cell eukaryotes….

- human genome -20 000-25 000 protein coding genes
- human microbiogenome - 2-20 millions protein coding genes
- all microbes constantly excrete/secrete small and large molecules and vesicles containing RNA, DNA, proteins - communication with neighbours and host – many of these molecules and vesicles can penetrate gut wall to bloodstream
- microbiome´s metagenome can change much faster than human genome – fast response
- microbiome = sophisticated additional organ (or set of organs)

Importance of human microbiota

- disturbance to human microbiota is connected to many diseases:
  - inflammatory bowel disease (ulcerative colitis and Crohn's disease)
  - diabetes
  - obesity
- microbes influence signal pathways/communication between digestive system and brain
  - neural, endocrine, immune signals

Human microbiota and obesity

- 2014 – 1,9 billion overweight people (600 mil. obese)
- GM of obese people shows **special metabolism** of carbohydrates and fats
- GM of obese people can digest substrates which human enzymes cannot
    - result is gain of energy, SCFAs – they influence metabolism of glucose, cholesterol, and fats in human tissues
- western diet (too much fats) reduce *Bacteroides* and promote *Firmicutes*
- GM activate bile acids and regulate metabolism (absorption of lipids)
- **germ free mouse** on diet rich in fats do not demonstrate obesity (urine, feaces)
- main thing is efficiency of utilization of energy from food

Facts about obesity

Worldwide obesity has nearly tripled since 1975.

In 2016, more than 1.9 billion adults, 18 years and older, were overweight.
Of these over 650 million were obese.

39% of adults aged 18 years and over were overweight in 2016, and 13% were obese.

Most of the world's population live in countries where overweight and obesity kills more people than underweight.

41 million children under the age of 5 were overweight or obese in 2016.

Over 340 million children and adolescents aged 5-19 were overweight or obese in 2016.

Obesity is preventable.

Human microbiota and obesity

Obesity is not only problem of weight, but is connected with higher risk of cancer (uterus, breast, cervix, large gut, rectum, throat, kidney, prostate, pancreas, etc.)

Obesity is connected with ready-to-cook meals (preserve chemicals, emulators)

Obesity is connected with higher incidence of Firmicutes and Actinobacteria and lower incidence of Bacteroides

Thank you for your attention