



# Microbiome 101

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Course lecture at Medical Microbiology II

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# Why should you care

	Annual cites from 2007 to 2019	Cites in 2007	Cites in 2019	Average annual percentage change	Correlations with microbiome papers in PubMed <sup>1</sup> (p-value)	Correlations with microbiome news published by SINC <sup>1,2</sup> (value)
Microbiome papers in PubMed	9297.0 (6063.3)	2600	21292	19.6%	-	<b>0.62 (0.023)</b>
Biomedicine papers in PubMed	1111673.6 (203280.1)	785933	1397557	4.9%	-	-
Microbiome/biomedicine in PubMed	0.8%	0.4%	1.4%	9.6%	-	-
Microbiome news in SINC <sup>2</sup>	8.1 (5.9)	0	9	24.8%	<b>0.62 (0.023)</b>	-
Biomedicine news in SINC <sup>2</sup>	582.1 (81.1)	666	447	-3.7%	-	-
Microbiome/biomedicine in SINC <sup>2</sup>	1.6%	0.2%	2.2%	19.5%	-	-
Total newspapers	4.6 (4.9)	2.3 (2.2)	7.8 (7.5)	13.9%	<b>0.88 (&lt;0.001)</b>	<b>0.66 (0.014)</b>
<b>Individual newspapers</b>						
<i>The New York Times</i>	10.3 (6.4)	5	20	16.0%	<b>0.83 (0.005)</b>	0.48 (0.095)
<i>The Times</i>	6.8 (4.4)	5	13	14.3%	<b>0.82 (0.005)</b>	0.47 (0.102)
<i>El País</i>	5.1 (4.0)	1	8	22.7%	<b>0.74 (0.004)</b>	<b>0.71 (0.006)</b>
<i>The Wall Street Journal</i>	4.1 (1.8)	2	4	2.9%	0.14 (0.652)	0.35 (0.236)
<i>Financial Times</i>	1.5 (1.6)	1	2	11.8%	0.39 (0.177)	<b>0.58 (0.038)</b>
<i>Expansión</i>	0.2 (0.6)	0	0	4.3%	0.41 (0.166)	0.11 (0.713)
<b>Country</b>						
USA	7.2 (5.6)	3.5 (2.1)	12.0 (11.3)	12.0%	<b>0.85 (0.002)</b>	<b>0.57 (0.039)</b>
UK	4.1 (4.2)	3.0 (2.8)	7.5 (7.8)	14.5%	<b>0.81 (0.001)</b>	<b>0.57 (0.042)</b>
Spain	2.7 (3.7)	0.5 (0.7)	4.0 (5.7)	23.1%	<b>0.75 (0.003)</b>	<b>0.68 (0.010)</b>
<b>Newspaper type</b>						
General newspaper	7.4 (5.4)	3.7 (2.3)	13.7 (6.0)	15.7%	<b>0.91 (&lt;0.001)</b>	<b>0.61 (0.024)</b>
Business newspaper	1.9 (2.1)	1.0 (1.0)	2.0 (2.0)	7.2%	0.39 (0.185)	<b>0.56 (0.043)</b>

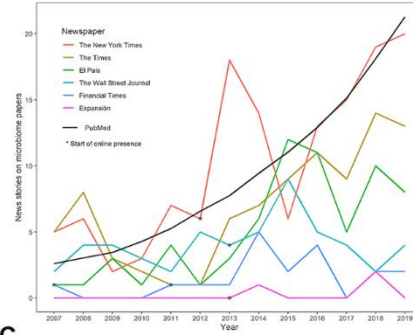
Mean followed by the standard deviation in parentheses is indicated for microbiome/biomedicine papers in PubMed, microbiome/biomedicine news in SINC and stories on microbiome papers in newspapers.

<sup>1</sup>The numbers showed the Pearson correlation coefficient.

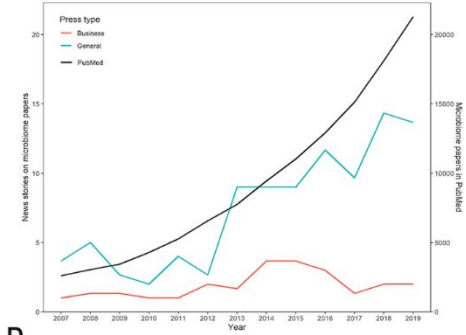
<sup>2</sup>News stories published by SINC were available from 2008 to 2018.

Significant p-values are highlighted in bold.

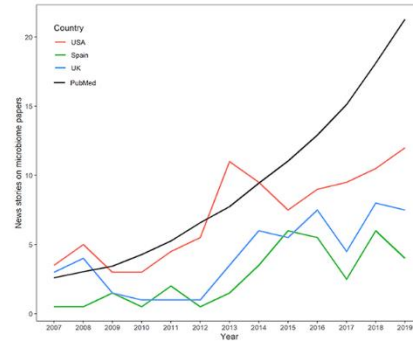
A



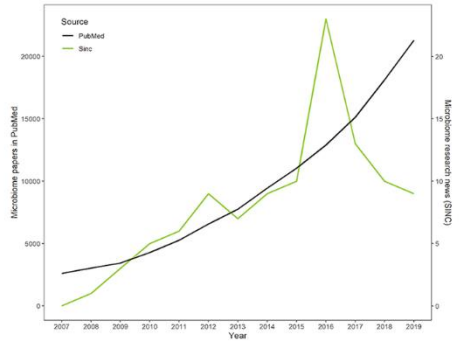
B



C



D



# Microbiome vs. microbiota

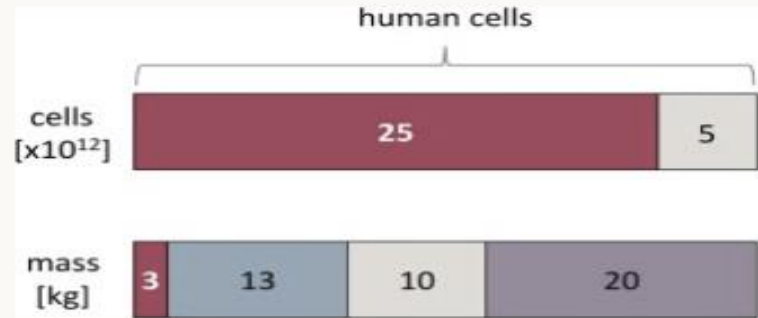


**Microbiome**  
= genome



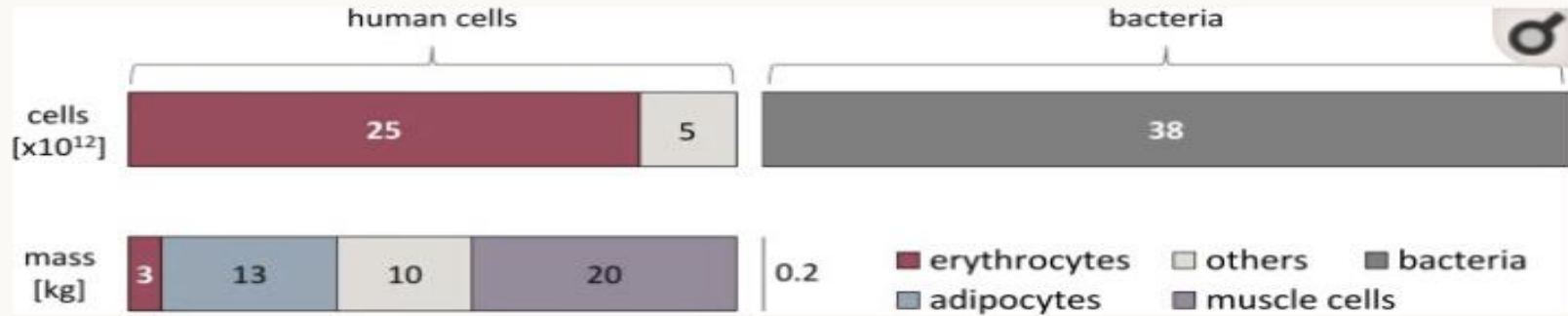
**Microbiota**  
= organisms

# We are outnumbered



Sender et al, PLOS, 2016

# We are outnumbered



Sender et al, PLOS, 2016

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

# Microbiome

IN NUMBERS

38

Trillion

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

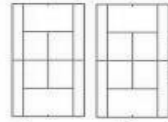
The human body has more microbes than there are stars in the Milky Way

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

ap 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

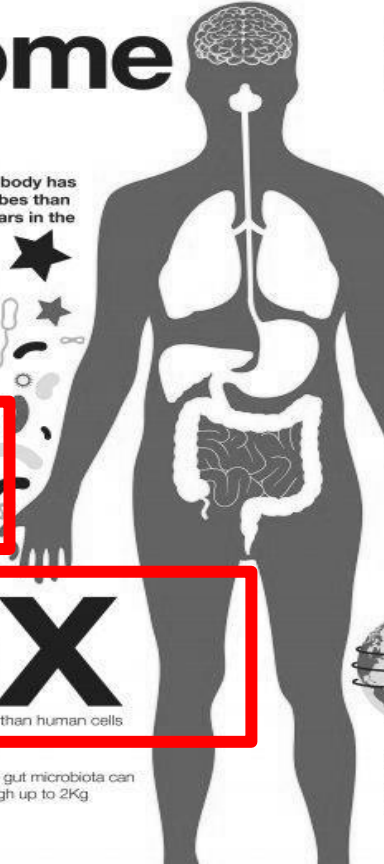
Viruses:Bacteria in the gut microbiota

2.5

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

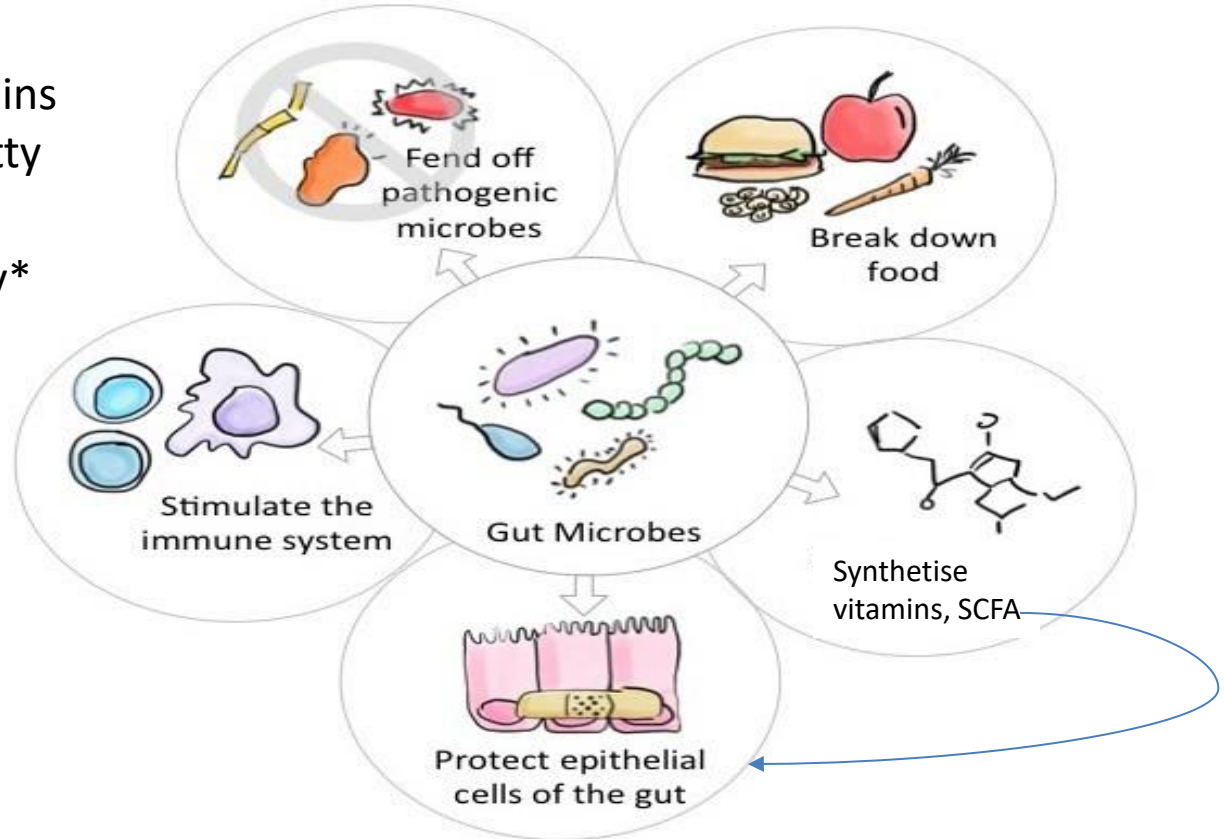


Each individual has a unique gut microbiota, as personal as a fingerprint

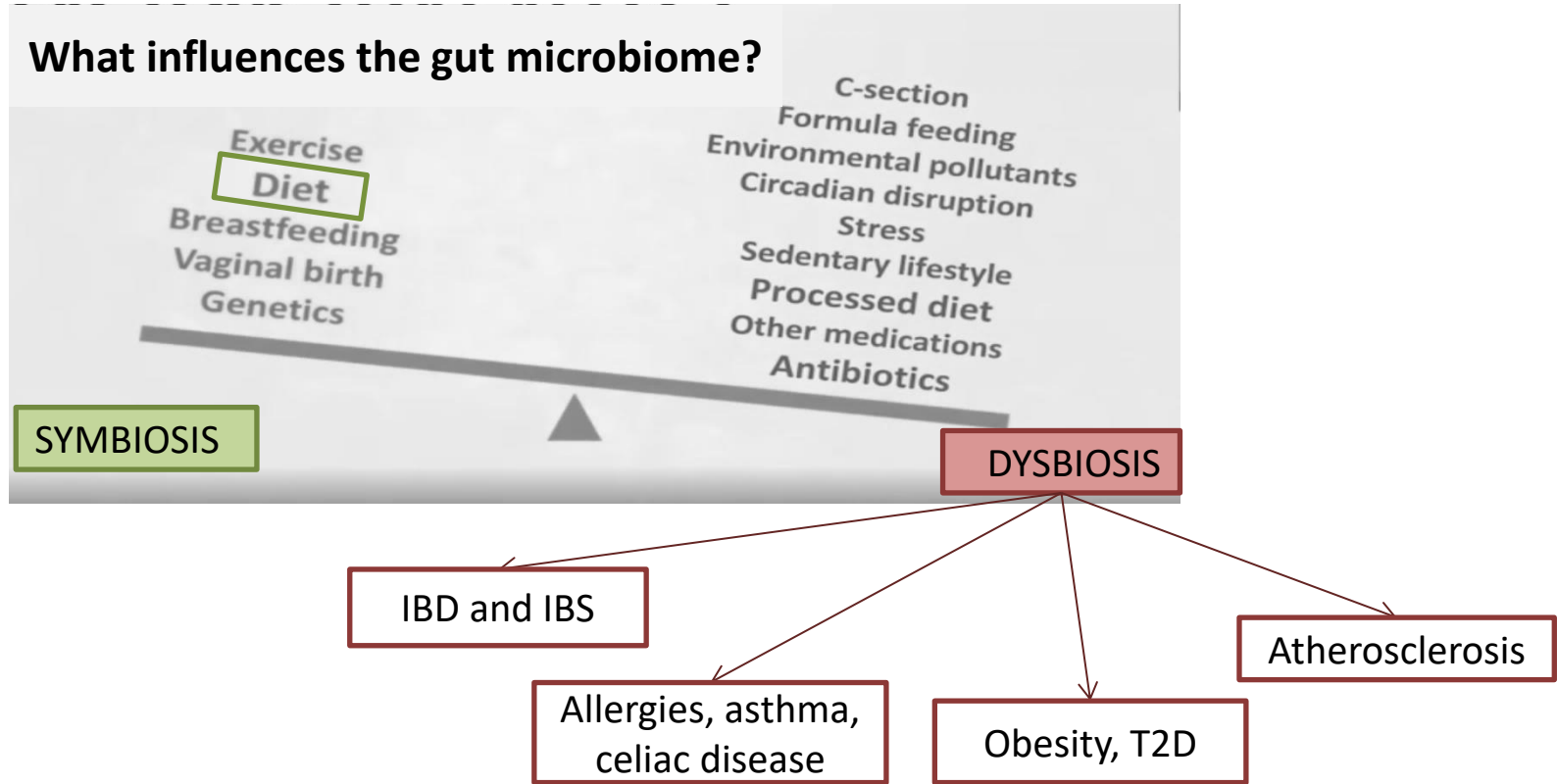


# Physiological role of the microbiome

- Digestion
- Metabolism (vitamins and short-chain fatty acids)
- Gut lineage integrity\*
- Immune system regulation
- Protection against infection

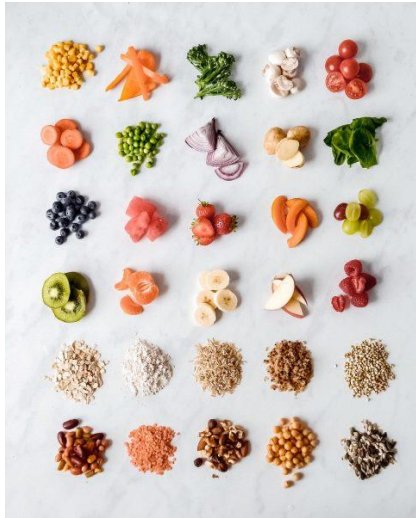


# Microbiome in health and disease



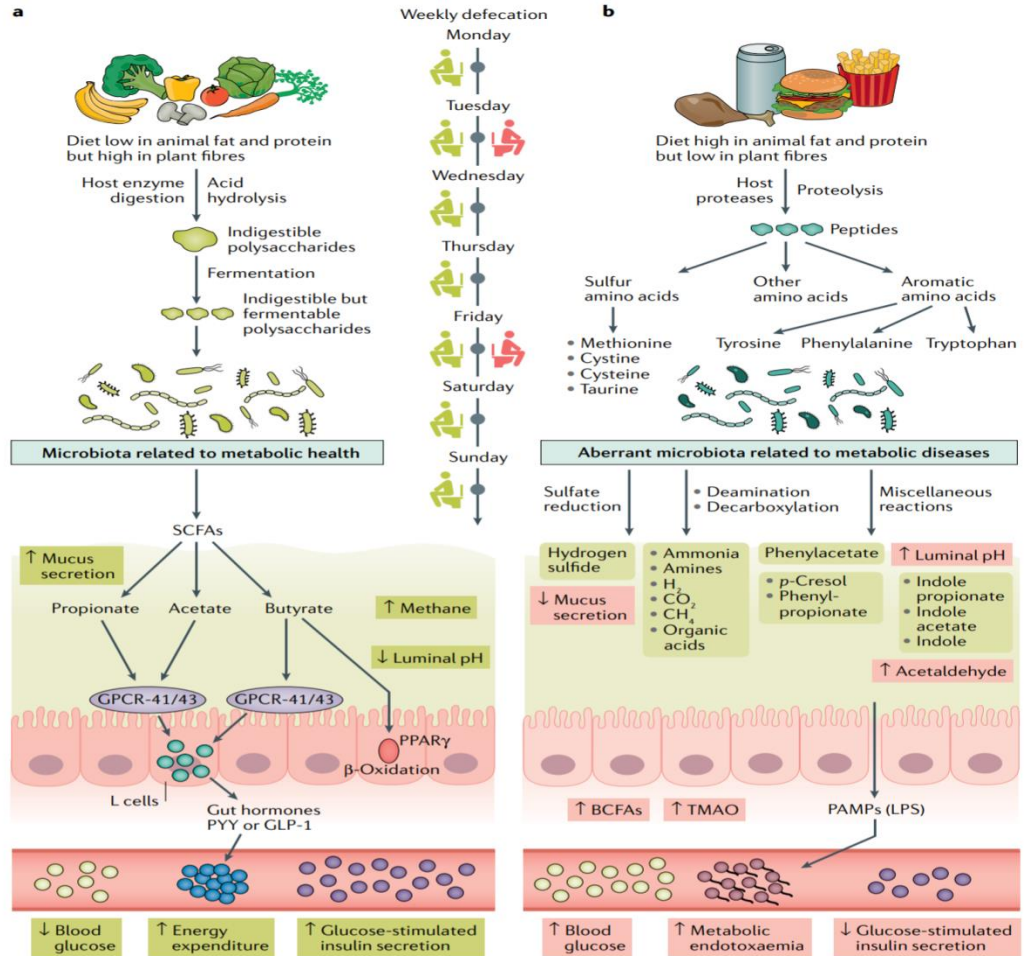


# What a good and bad diet does?



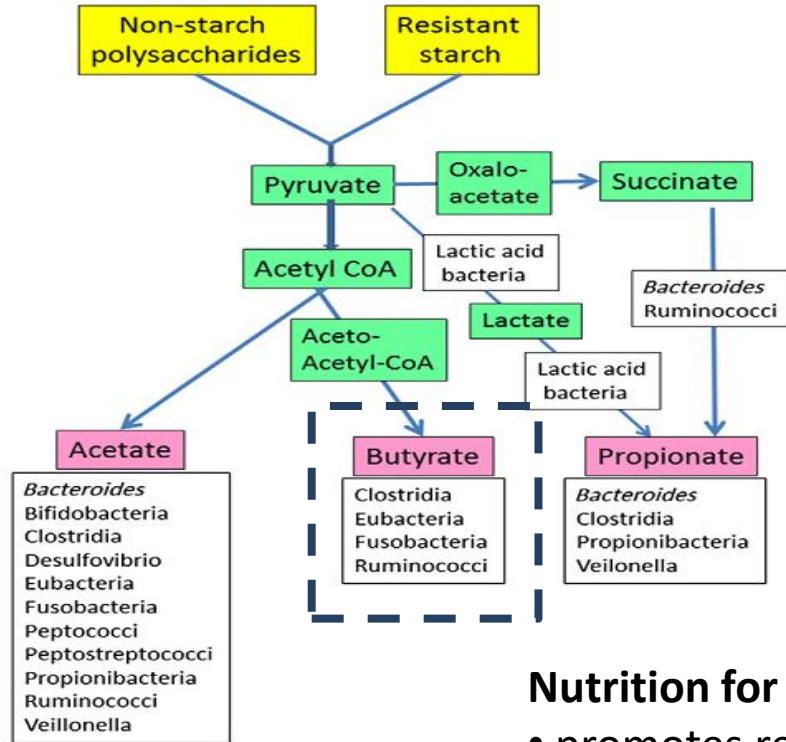
Thirty different plants per week

(Knight et al, American Gut Project, 2012)



Fan & Pedersen; *Nature Reviews Microbiology*, 2020

# Short chain fatty acids (SCFA)



## Nutrition for enterocytes:

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

# Gut microbiome inhabitants

## Main Phyla

## Class

## Example Genera

*Actinobacteria*

*Actinobacteria*

*Actinomyces; Bifidobacterium*

*Bacteroidetes*

*Bacteroidia*

*Bacteroides; Prevotella; Alistipes*

*Firmicutes*

*Bacilli*

*Bacillus; Staphylococcus*

*Enterococcus; Lactobacillus; Lactococcus; Streptococcus; Leuconostoc*

*Clostridia*

*Clostridium; Coprococcus; Roseburia; Faecalibacterium; Ruminococcus*

*Negativicutes*

*Veillonella*

*Proteobacteria*

*Epsilonproteobacteria*

*Helicobacter; Campylobacter*

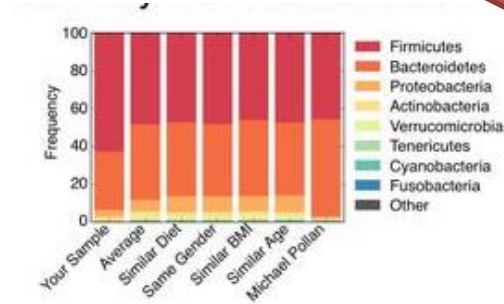
*Gammaproteobacteria*

*Citrobacter; Escherichia; Shigella; Klebsiella; Providencia ....*

*Verrucomicrobia*

*Verrucomicrobiae*

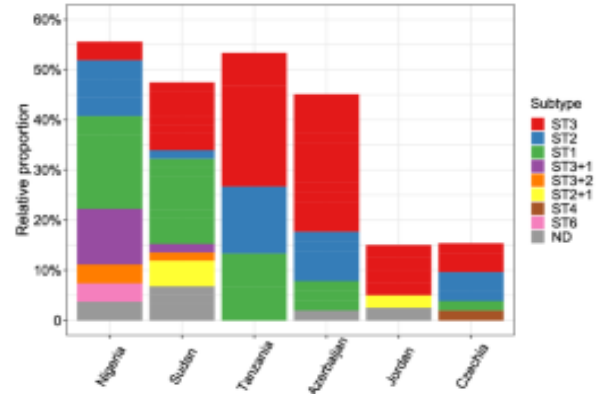
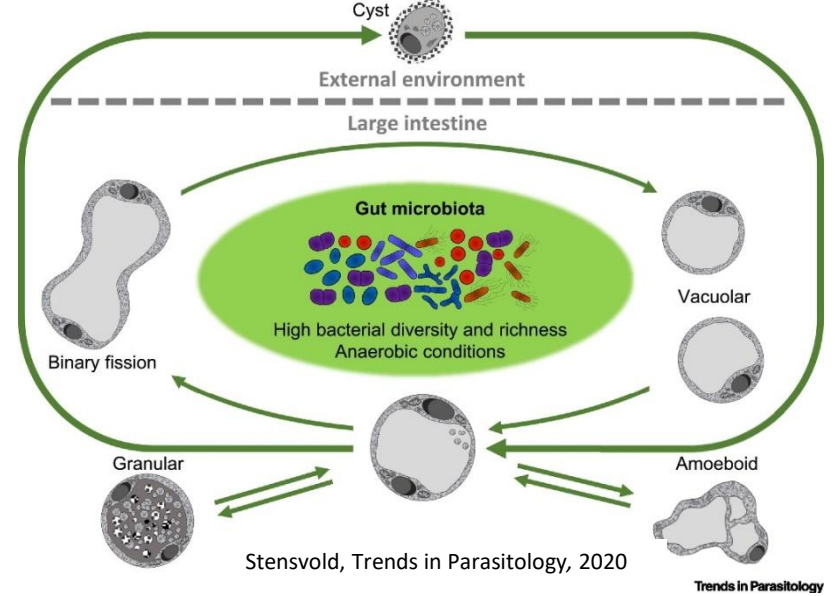
*Akkermansia*



90%

# *Blastocystis* sp.


- Most abundant eukaryotic organism in human gut
- Marker of high bacterial diversity
- Prevalence lower in developed countries (30-50%), much less in gut diseases
- Classified in subtypes (ST1-ST22)



Cinek et al, *Parasites and Vectors*, 2021

### Stool collection

Stored at -80°C




### DNA Extraction

DNA stored in -80°C




### 16S rDNA profiling

PCR for 16S rDNA



Mass sequencing of this gene



Group reads by similarity, count them


Classify taxonomy

```

>OTU1: AAGCATATGCTATGATCGATCATGACT
>OTU2: CATGATCTGACTATTATTCGCGATTG
>OTU3: GCGATATTCGATCTATTTCGATGCGGAT
.....
>OTU1: Firmicutes; Clostridia; Clostridium piliforme
>OTU2: Firmicutes; Clostridia; Ruminococcus bromii
>OTU3: Firmicutes; Bacilli; Leuconostoc
    
```

### Metagenomic sequencing

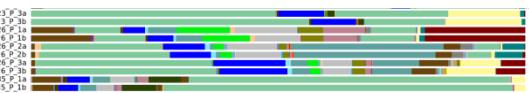
Randomly fragment total DNA



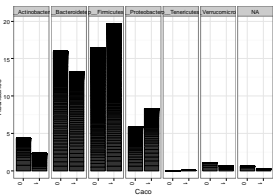
Sequence, assemble genes

### Data analysis

Analyze composition



Compare cases with controls



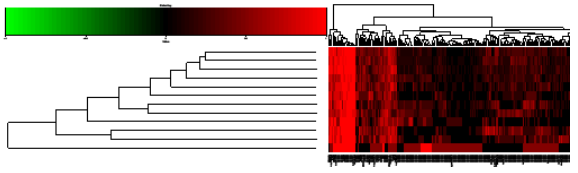
Case	Actinobacteria	Bacteroidetes	Firmicutes	Proteobacteria	Planctomycetes	Nonclassical	NA
23*_30	~1	~1	~1	~1	~1	~1	~1
23*_10	~1	~1	~1	~1	~1	~1	~1
26*_10	~1	~1	~1	~1	~1	~1	~1
26*_20	~1	~1	~1	~1	~1	~1	~1
26*_30	~1	~1	~1	~1	~1	~1	~1
26*_10	~1	~1	~1	~1	~1	~1	~1
35*_10	~1	~1	~1	~1	~1	~1	~1

### Classify genes by function

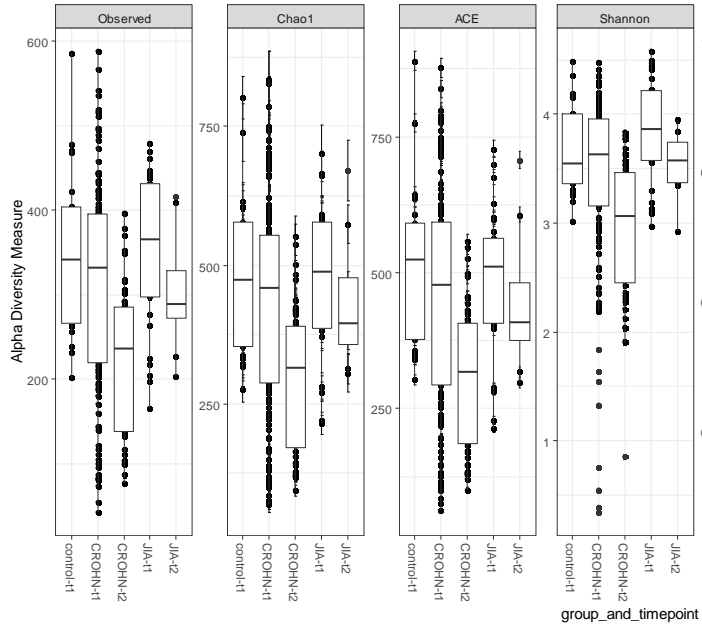
- > glucosidase
- > lambda phage capsid
- > lactase
- > cable pilus
- .....

Assess functional capabilities of the microbiome

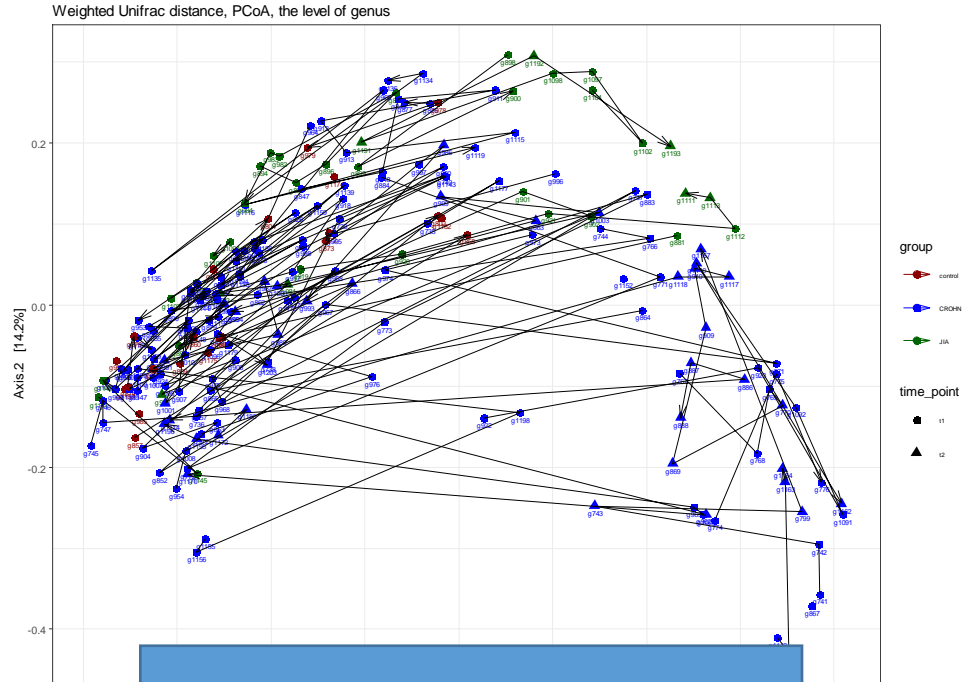
Compare cases with controls



# Alpha and beta diversity



In one sample



Between samples

# What is a „good“ and a „bad“ result?

## **GOOD**

Higher alpha diversity (300-1000 species)

Anaerobic environment (e.g. very few *Proteobacteria*)

More SCFA producers

*Blastocystis* positive

## **BAD**

Lower alpha diversity (ca less than 100 species)

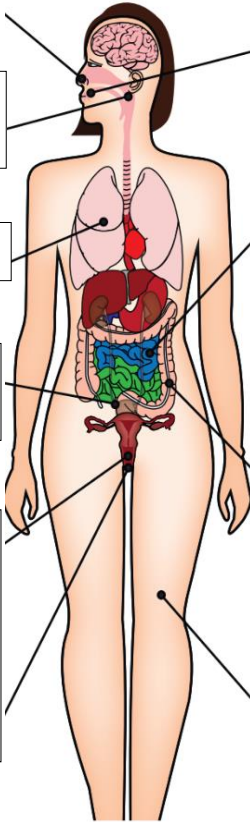
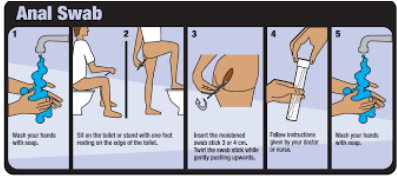
Many facultative anaerobes (e.g. more *Proteobacteria*)

Less SCFA producers

*Blastocystis* negative

# Keep calm when you see:

CoNS in only 1/6 bottles



CoNS, *S. aureus*, diptheroids

Diptheroids, viridans strep, oral neisseria

(Viridans strep)

CoNS, diptheroids, enterococci (10e3 and lower in urine)

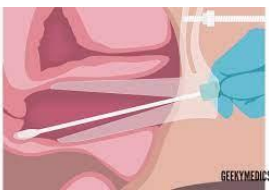
Lactbacilli, corynebacteria, CoNS, streps

Viridans streps, oral neisseria, *Veillonella*

Anything apart from:

- *Campylobacter*, *Salmonella*, *Yersinia*
- *C. difficile* and *H. pylori* (both in stool)
- Parasites (*Cryptosporidium*, *Entamoeba histolytica* e.g.) but not *Blastocystis* and maybe not *Dientamoeba*

CoNS, *S. aureus*, diptheroids, *C. acnes*, micrococci



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# Watch out when you see:

## Anything in a „sterile“ sample:

- Tissues
- Heart valves
- Blood cultures (apart 1/6 CoNS)
- Joint aspirate
- Cerebrospinal fluid

## Among others:

- More than  $10^3$  CFU from suprapubic puncture
- STD pathogens in children in urine
- *E.coli* in toddlers from stool

## When patient/parents ask about the gut microbiome

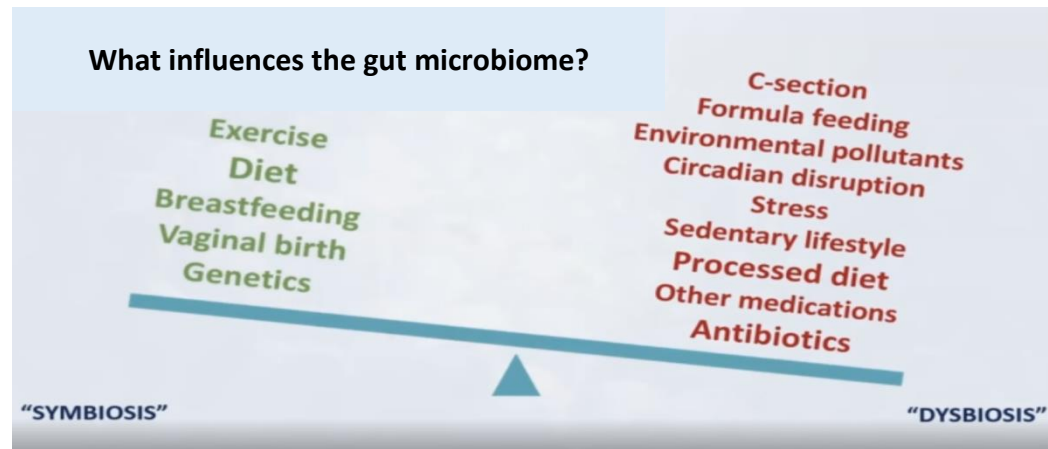
### Tell them to:

- Eat diversity of plants
- Sleep well, exercise, breast-feed and be outside

**Consulting results:** check the diversity and anaerobes

## What can you do as a physician

- Tell them the same without asking for it
- Prescribe ATB wisely
- (Do not treat Blastocystis in asymptomatic patient)



Keep calm when you see physiological microbiota

- But feel free to call us anyway ;)

# Take-home message

1. You are a **superorganism** (cell-wise: 1.3x more microbial)
2. Fiber rich food are food for the gut bugs and they produce SCFA's which are a food enterocytes and keeps the gut integrity
3. Main phyla in the gut are *Firmicutes* and *Bacteroides*
4. Tell patients to eat plenty of whole-food plants, exercise, breastfeed. Prescribe ATB's wisely
5. Learn the physiological microbiota findings to be calm when you see the results