The metabolic potential of microbial communities

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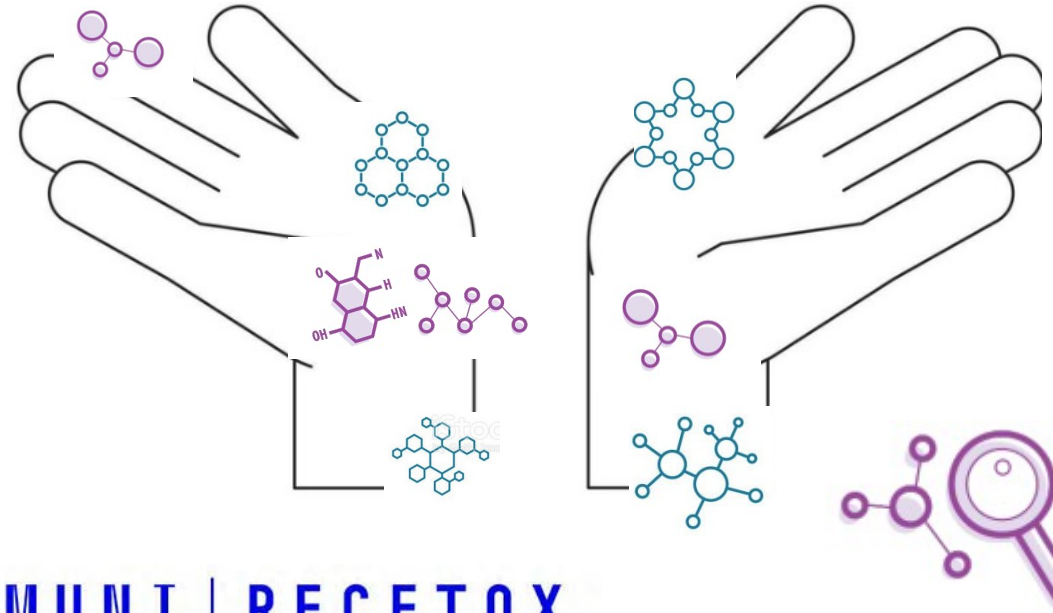

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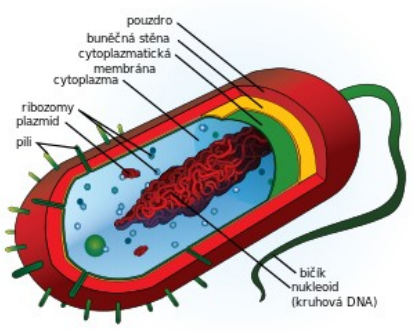
Microbiome Metabolome Integration
Why this research line?

The Invisible Us – The Human Microbiome in Health and Disease, https://dx.doi.org/10.31487/sr.blog.07

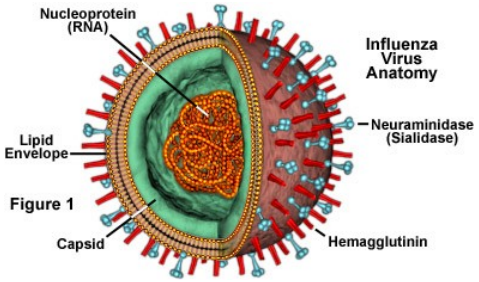
Microbiome Metabolome Integration Platform (MMIP): a web-based platform for microbiome and metabolome data integration and feature identification., https://doi.org/10.1101/2023.04.04.535534

Microbiome

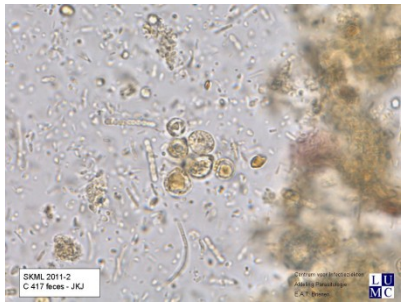
- Microbiome - a community of microorganisms that can usually be found living together in a given environment
- Microorganism - a single-cell organism of microscopic size
  - Bacteria
  - Viruses
  - Fungi (Brewer's yeast is a eukaryote belonging to this kingdom)
  - Algae

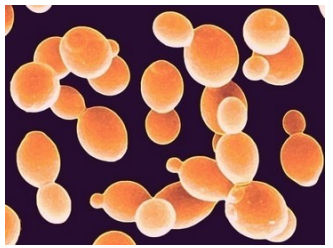


http://www.wikiskripta.eu



http://aboutviruses.weebly.com





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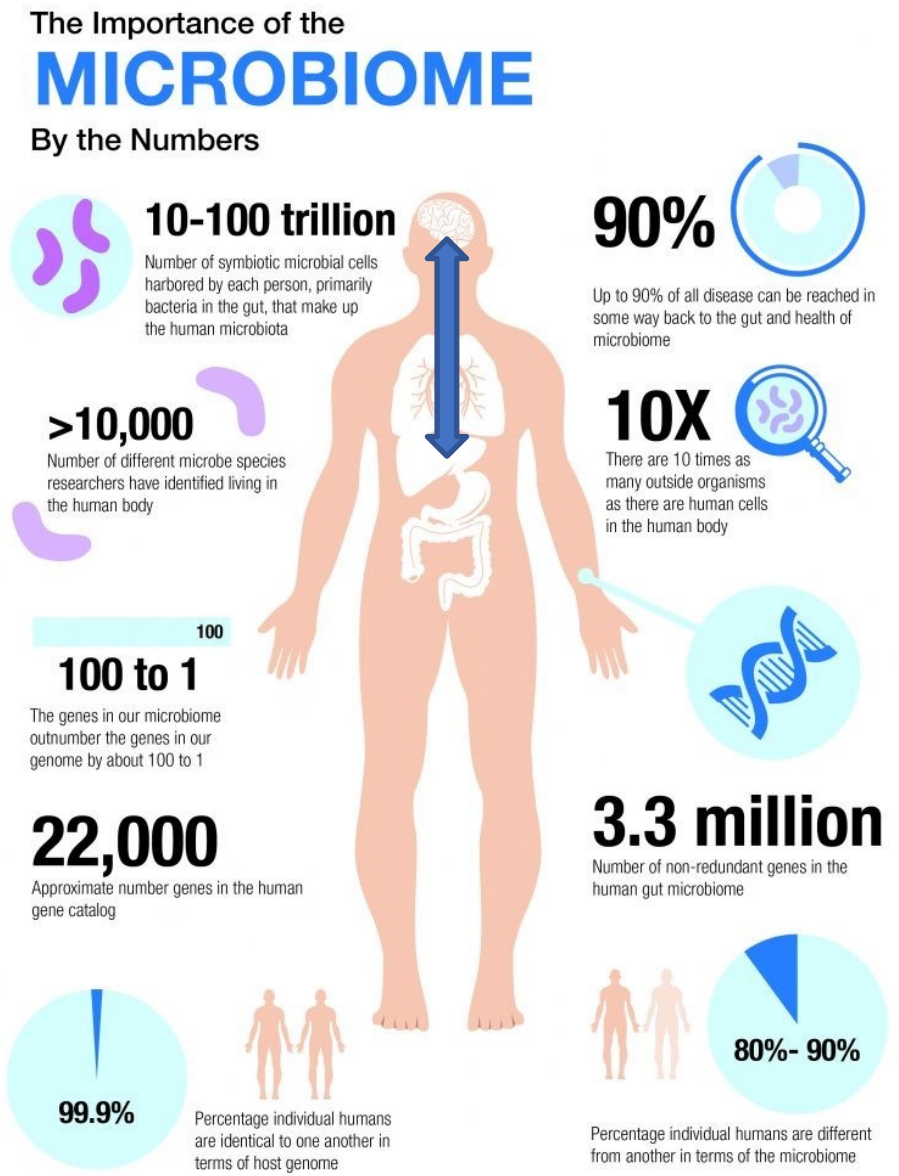
The human microbiome

- We have more bacteria in our body than our own cells
- Bacterial genes outnumber human genes 100:1
- More than 1000 species of bacteria live in the intestine
- Based on the microbiome, a person can be identified in a similar way to fingerprints
- Each person has an individual composition of the intestinal microbiome, it differs from 80-90%
- **Gut microbiome** and discovery of **Gut Brain axis**: the two-way biochemical signaling that takes place between the gastrointestinal tract (GI tract) and our central nervous system (CNS)



The Invisible of us https://dx.doi.org/10.31487/sr.blog.07

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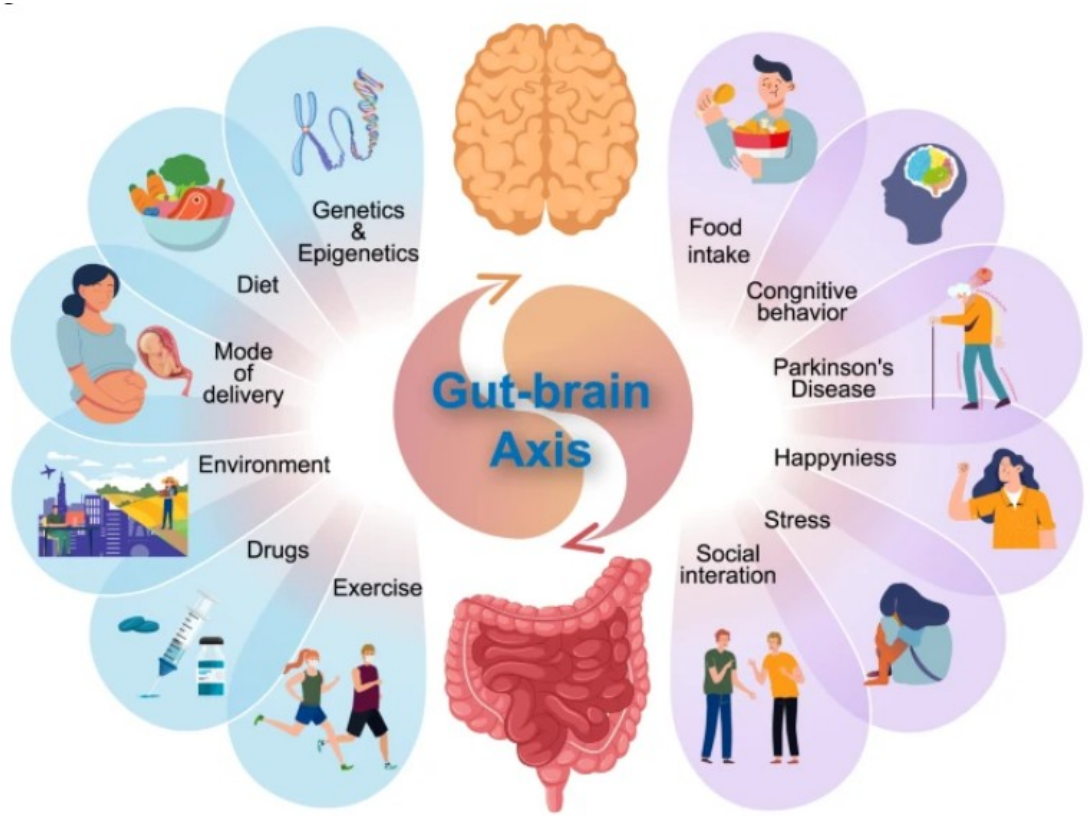
Microbiome in healthy status: contributing internal and external factors



Microbiota in health and diseases, https://www.nature.com/articles/s41392-022-00974-4

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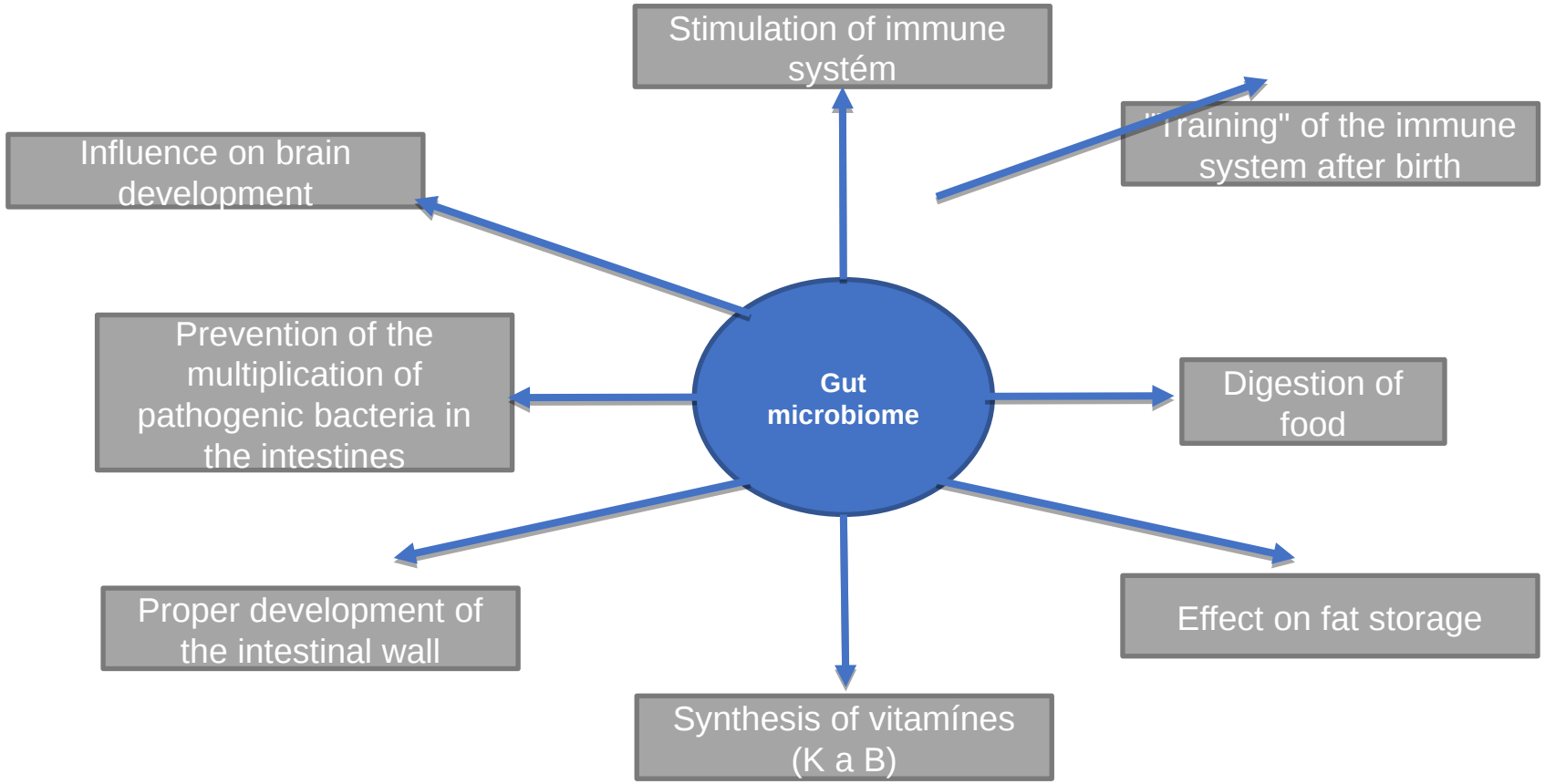
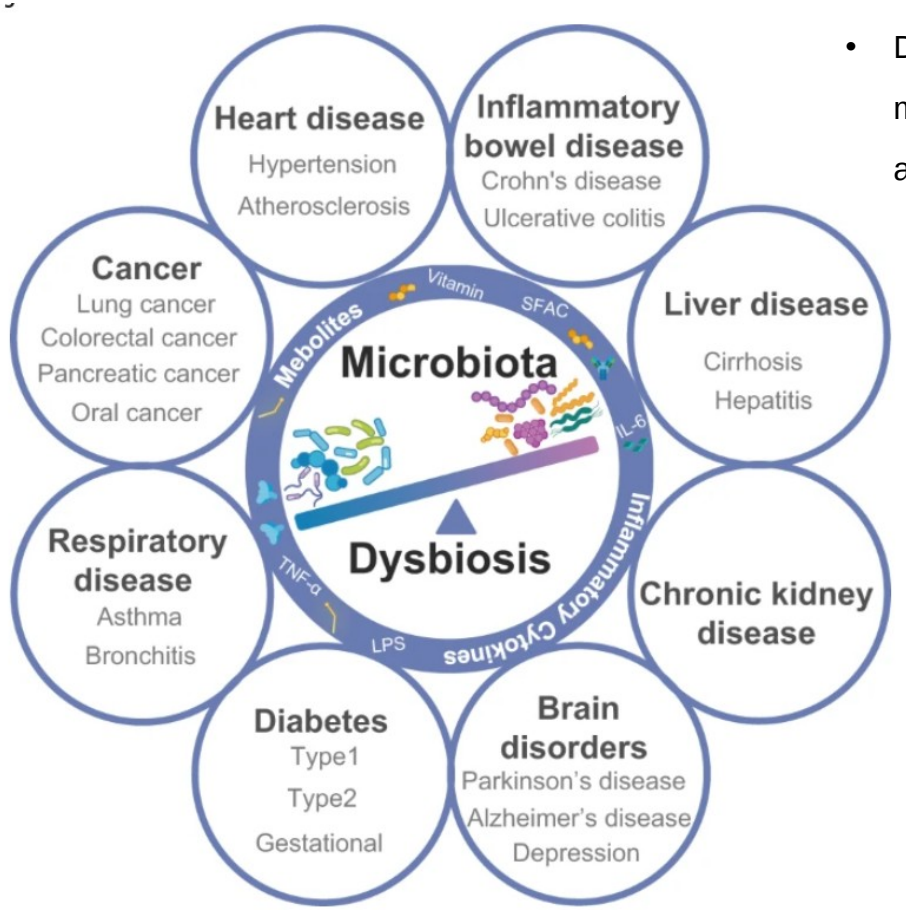
How does the microbiome affect health?



When something doesn't work...



- Dysbiosis, a state of disruption of the balance of the microbiome and resulting changes in its composition and function

Microbiota in health and diseases, https://www.nature.com/articles/s41392-022-00974-4

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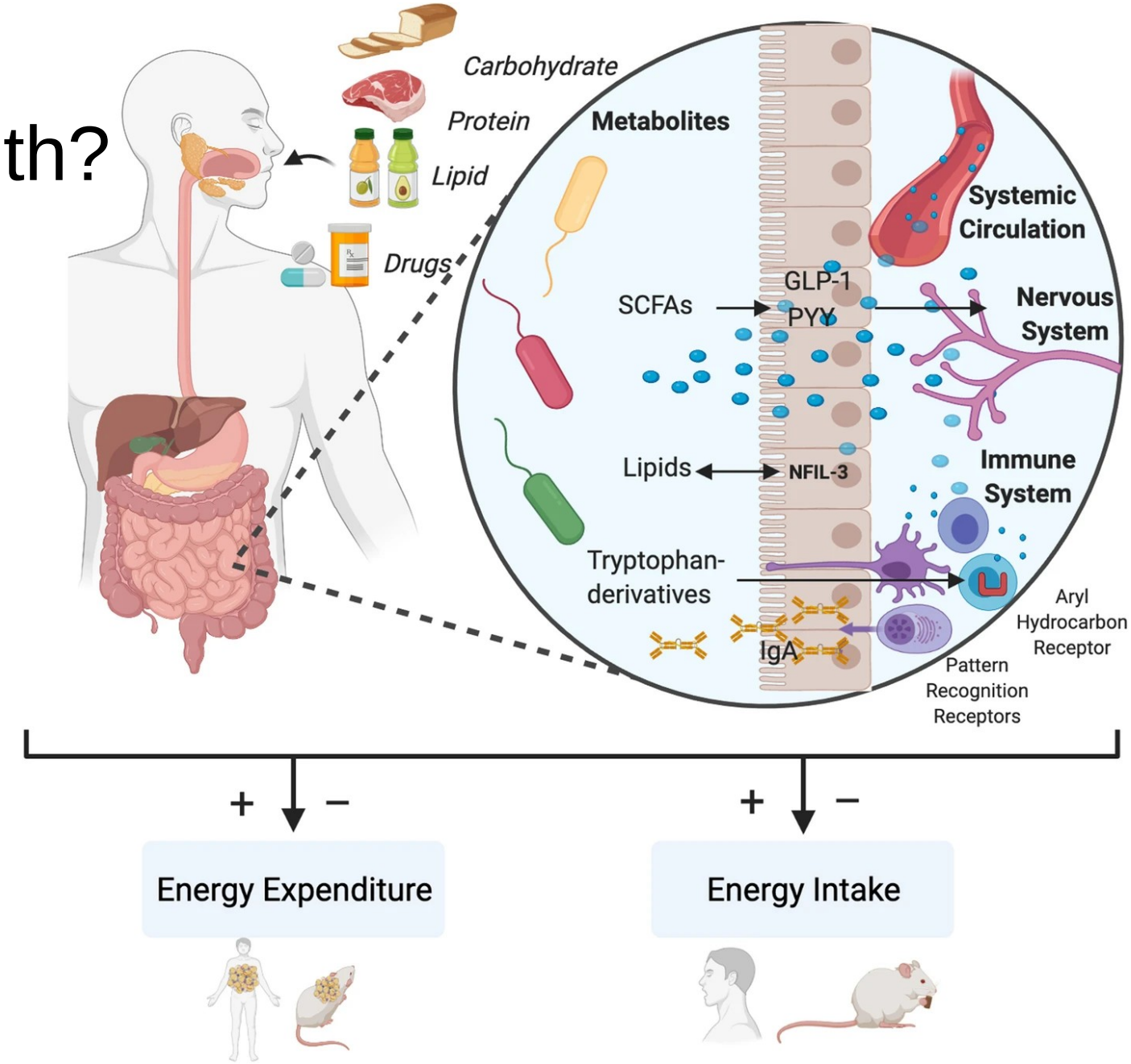
How does the microbiome affect health?

- By its metabolic activity:
- it is processing something
- It creates something



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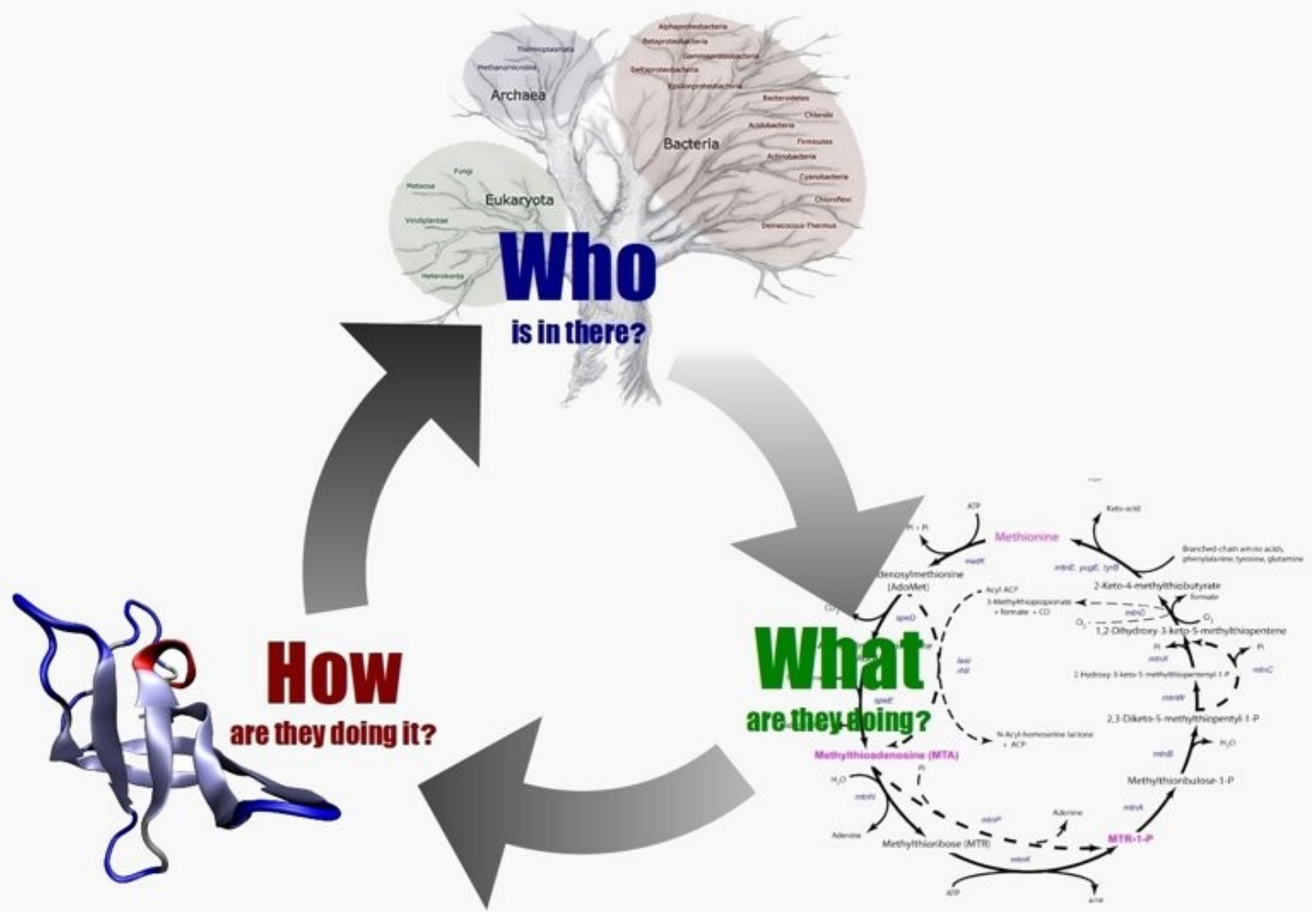
Metabolic control by the microbiome | Genome Medicine | Full Text (biomedcentral.com)

Microbiome research in health asks 3 basic questions



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How to answer these questions? -”Who”

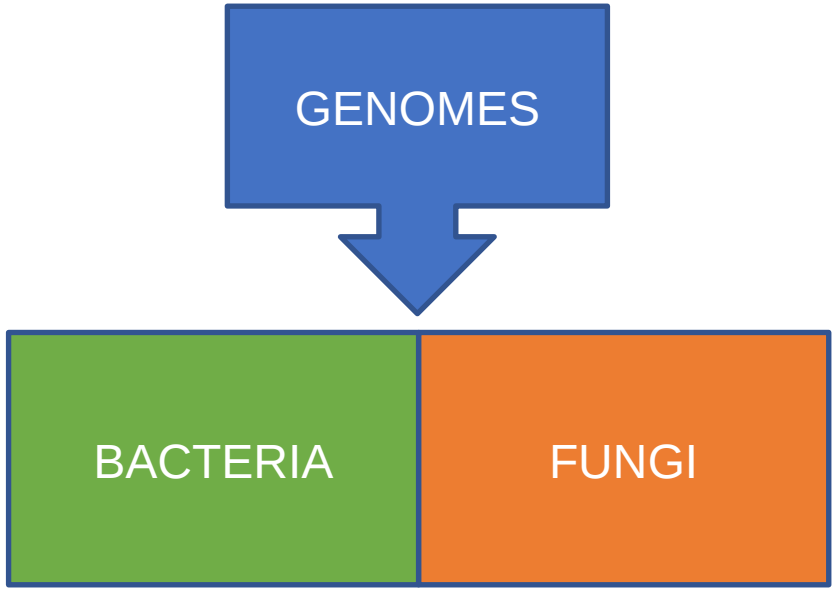
- Simple - we find out what bacteria are in the gut -> we make a genotype -> we estimate the functions

How to answer these questions?

- Simple - we find out what bacteria are in the gut -> we make a genotype -> we estimate the functions
- Traditional procedures - cultivation?
- The problem: most bacteria in the gut are not culturable

Metagenomics

- Study of the genomes of all microorganisms in the sample (soil, water, skin smear, feces, tumor...)

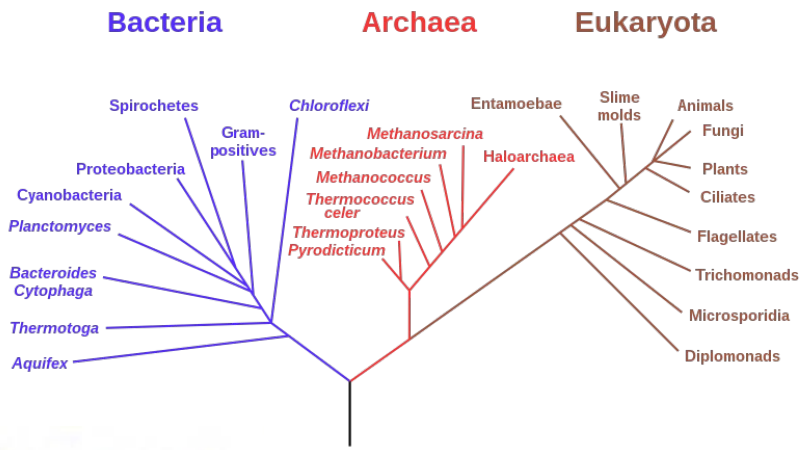


How to explore the metagenome?

Marker metagenomics
(targeted sequencing)

Amplicons corresponding to the whole (or parts) of genes of so-called phylogenetic markers (16S rRNA, rpoB...) are isolated, extracted and sequenced.

Marker genes are used as “species-specific taxonomic barcodes” – a rapid estimate of taxonomic composition



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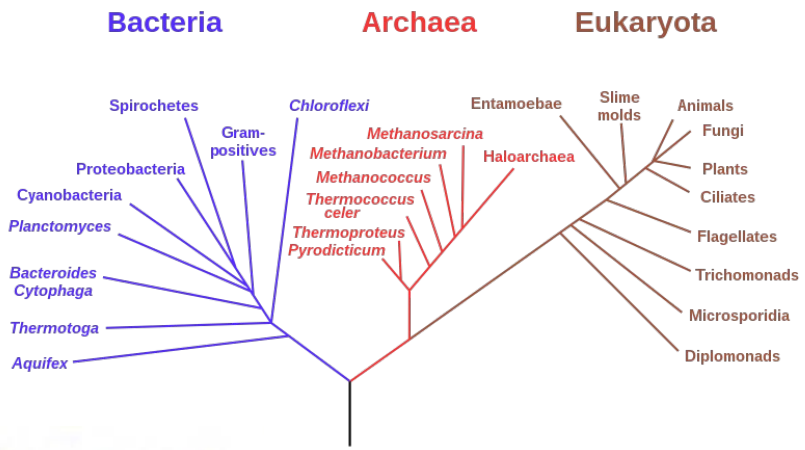
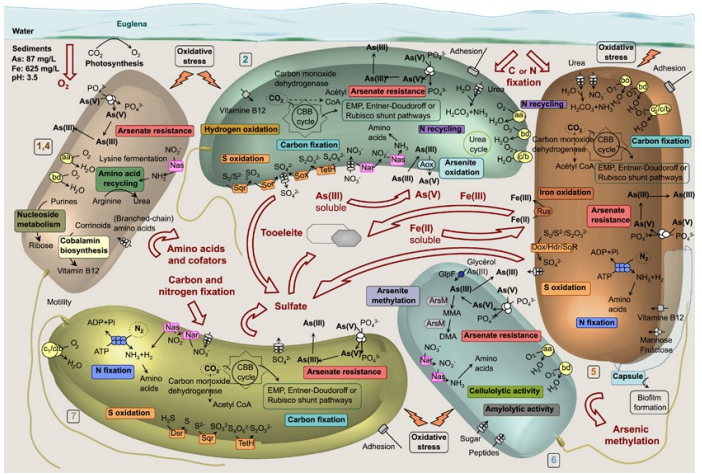


Shotgun metagenomics (whole genome sequencing)

The entire genome of the microbiome in the sample is extracted and sequenced. It provides insight into the taxonomic composition and function of the microbiome.



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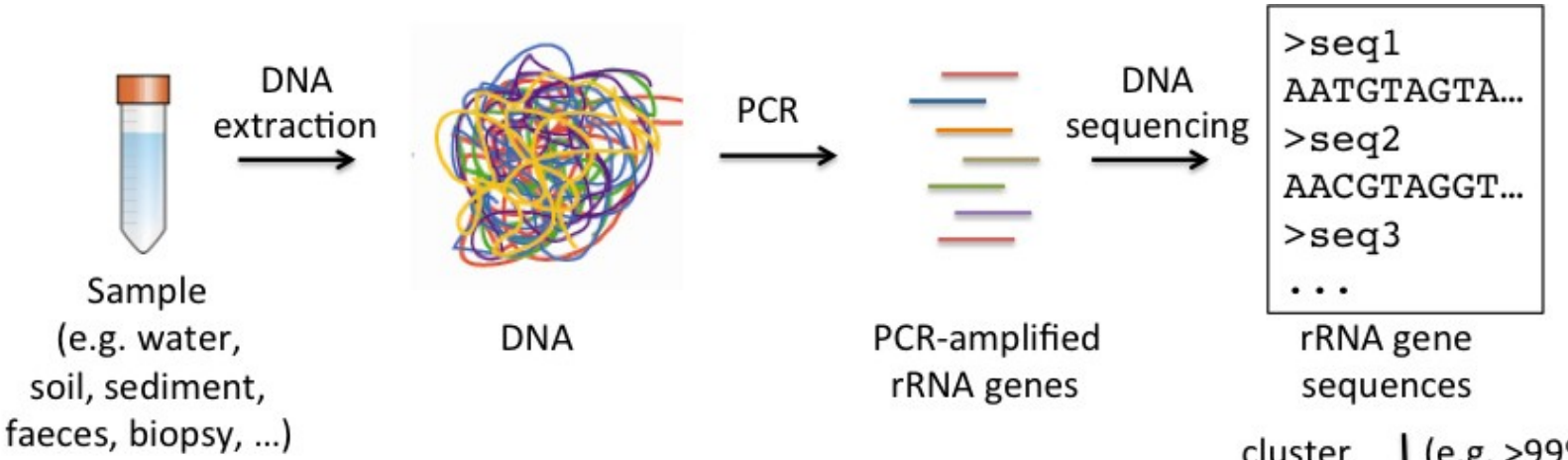
Marker metagenomics (targeted sequencing)



Taxonomic composition

OTU	Species	Sample1	Sample2	Sample3
1	E.coli	17	0	335
2	S.aurus	231	11800	45
3	unknown	30	0	0
…	…	…	…	…

Counts of OTUs per sample

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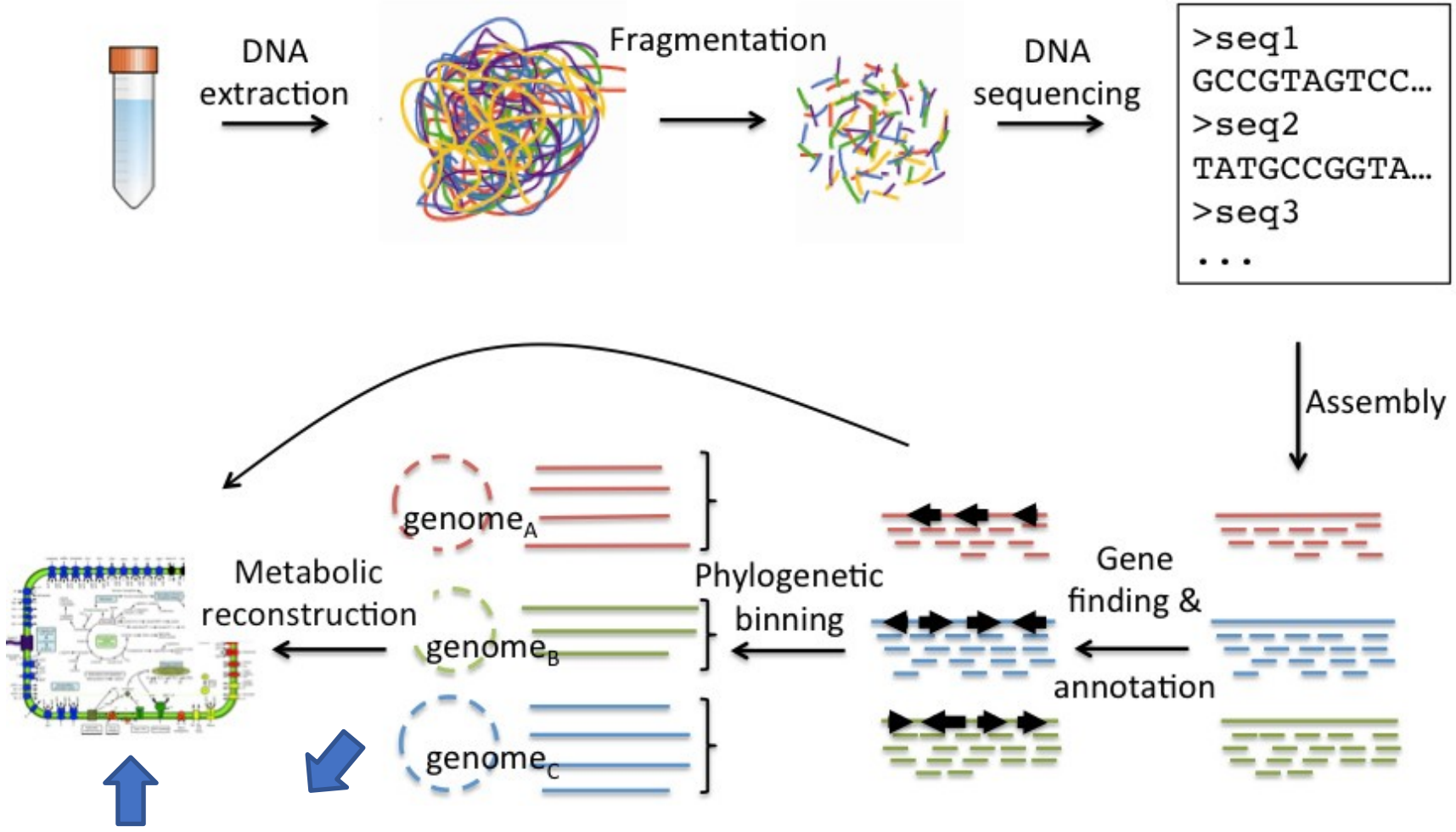
Shotgun metagenomics (whole genome sequencing)



How do we get answers for “what” and “How”.?

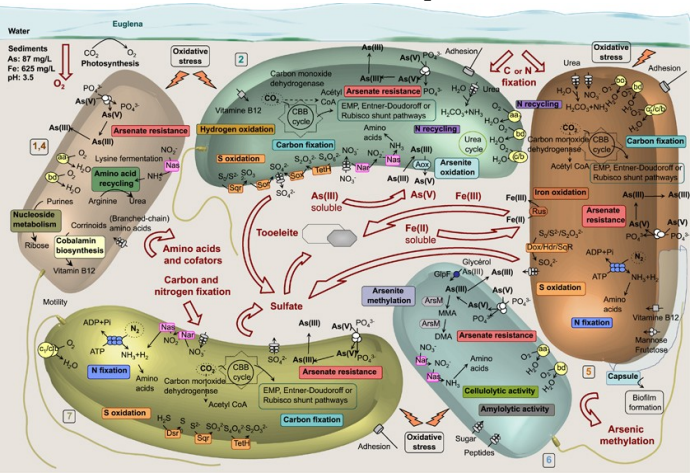
By sequencing we will find out what the microbiome is and its genome

KEGG, REACTOME, UNIPROT, ...

We find out the function of genes from web knowledge base (knowledgebases) about genes, their functional products and their involvement in molecular pathways

application of special bioinformatics tools:
PICRUST + PRMT, METAPHLAN,
....

We have information about the composition and functional POTENTIAL of the microbiome (metabolic pathways and metabolites)



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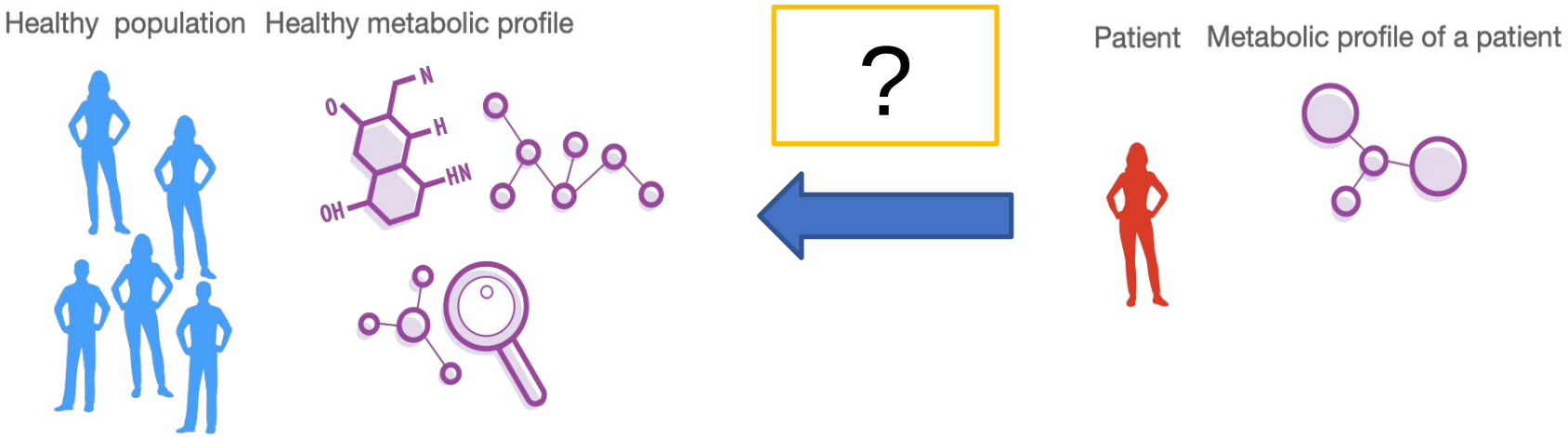
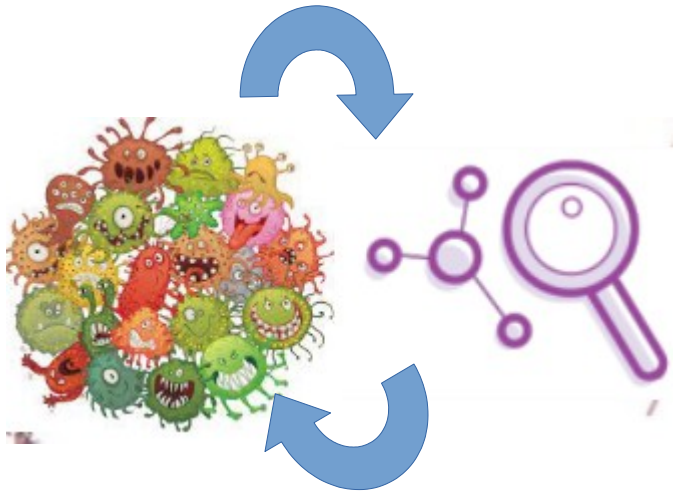
How to bring a sick person closer to a healthy person?

Hypothesis: the microbiome affects health through metabolites => changing the microbiome of a sick person can help to change his metabolites and thus his health status

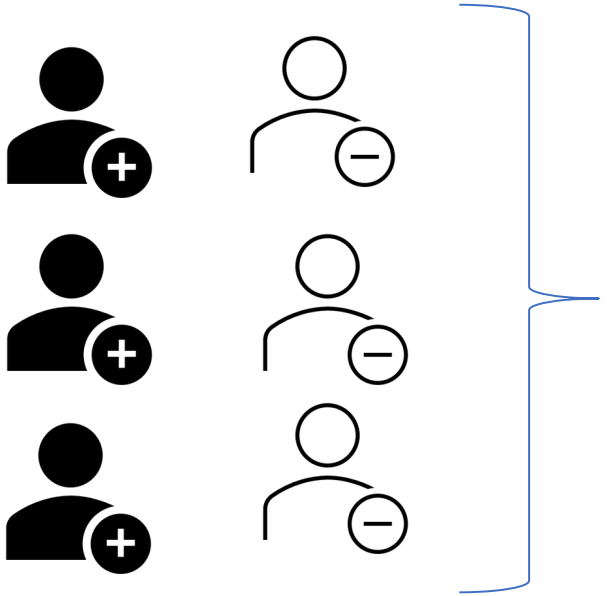


But what do we not know (gap of knowledge)?

- How to use a list of differently abundant bacteria or differently expressed metabolites to treat a patient - to change their individual microbiome...



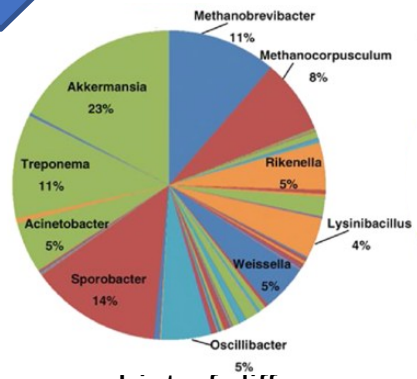
A typical data integration strategy in Microbiome Metabolome Integration



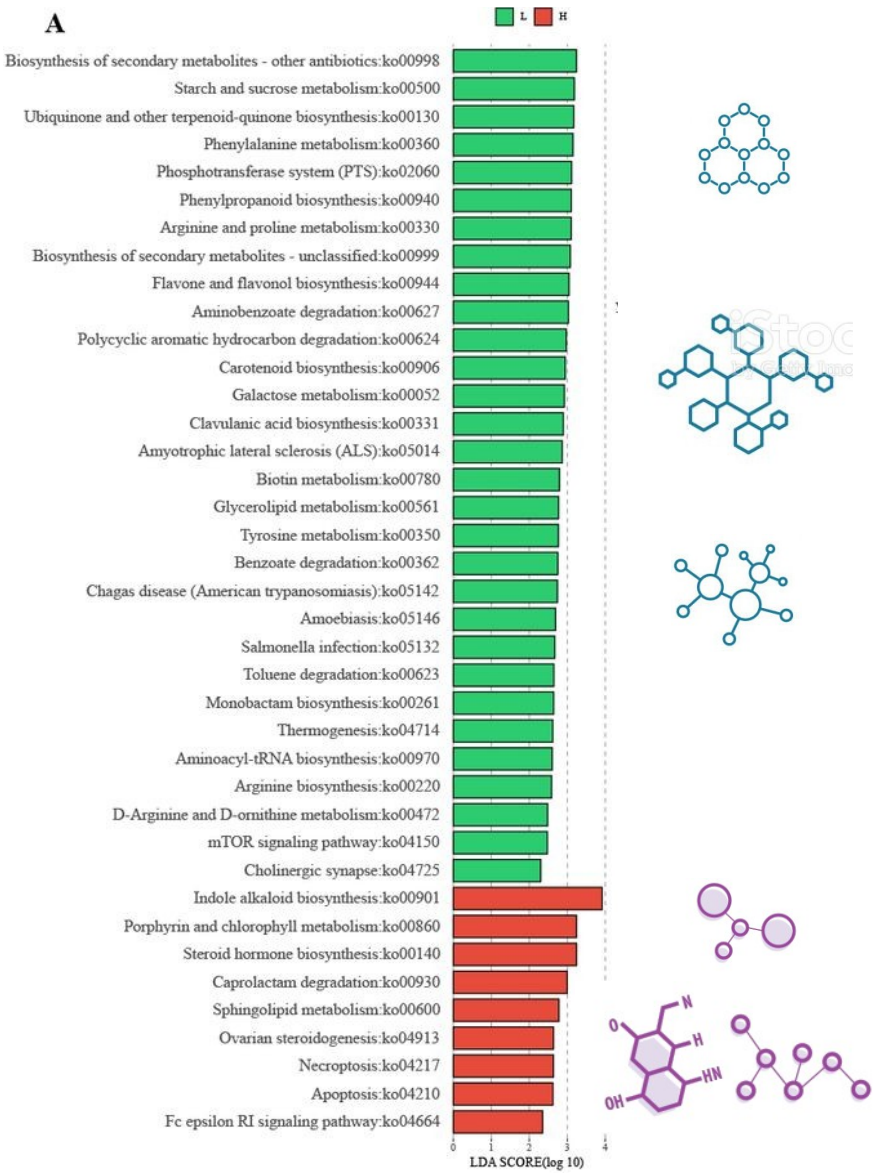
Healthy population and diseased population

Metabolic and microbial profiles of individuals

Comparison between groups



List of differences in microbiome composition



List of different metabolic pathways (Picrust)
List of different metabolites (PRMT method)

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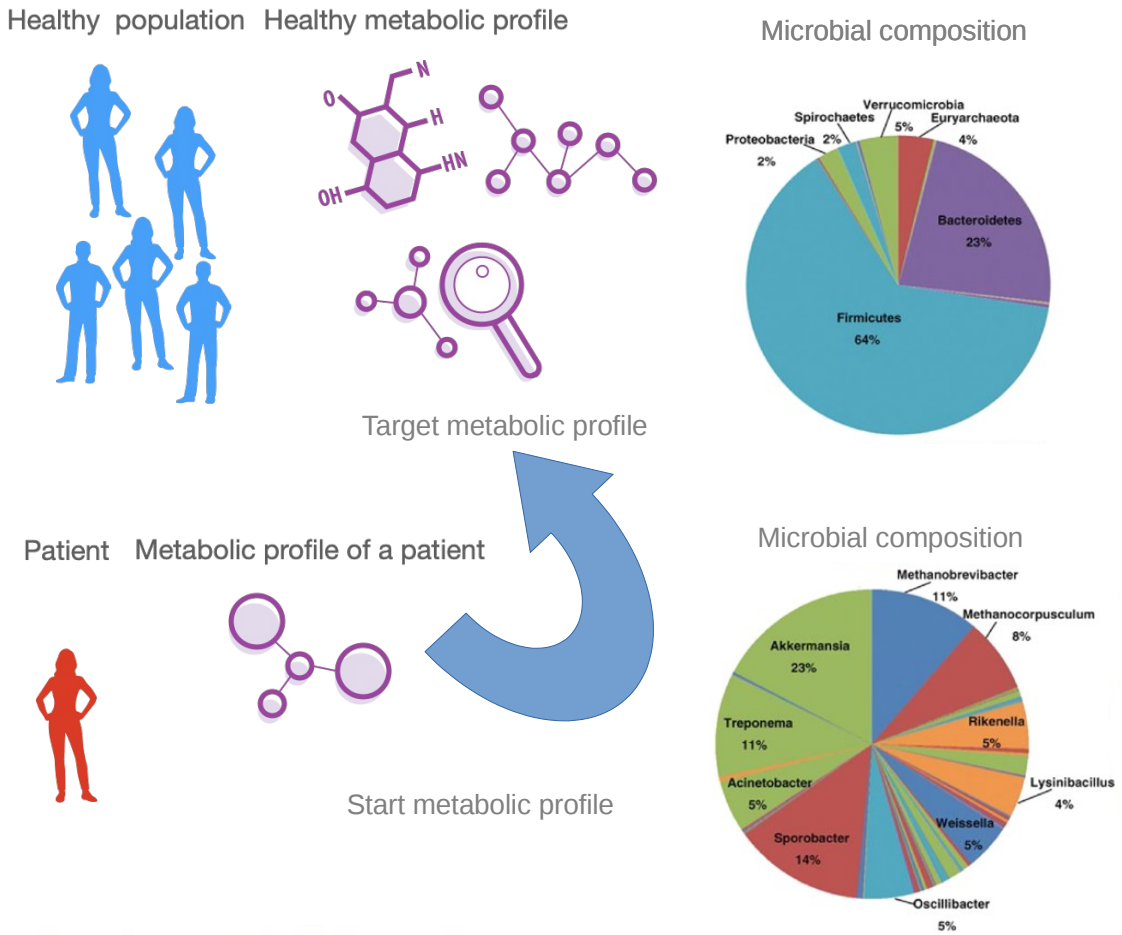
What do we need?

A method that estimates the microbial composition based on the desired (or target) metabolic profile
The method can be implemented to end up with a software tool.



What is the **target metabolic profile** of a healthy individual?

Thanks to our clever experimental design we have also collected data from healthy individuals...

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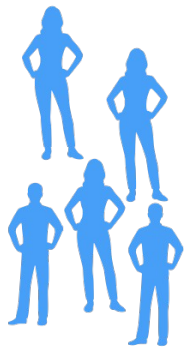
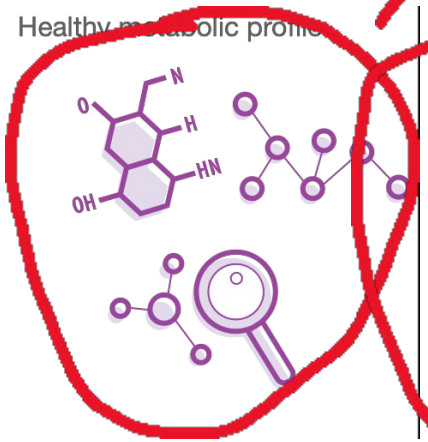
We have microbial composition of healthy people. What do we need?

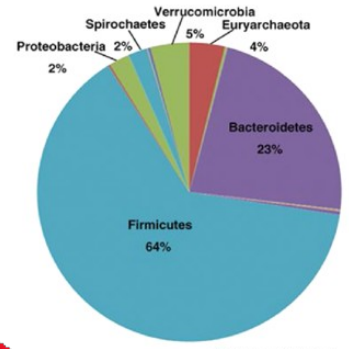
A method that estimates the microbial composition based on the desired metabolic profile

Healthy population Healthy metabolic profile





Microbial composition



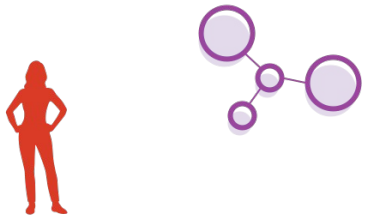
"Treponema"=11.02
"Oscillobacter"=3.05
"Bacillus S."=7.18
"Clebsiells" =1.29
…
…
…
"E.Coli" =6.77

which microbes produce which metabolites?

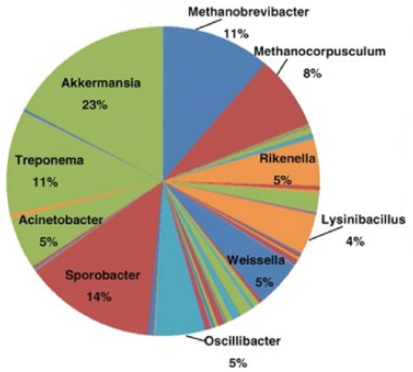
	Metabolity			
A type of microbe	M1	M2	M 3	M4
Methanobrevibacter	2	0	1 0	5
Oscllibacter	5	0	3	0
Akkermansia	0	4	1 2	0
Treponema	1	6	0	0

Metabolity	Skóre
M1	50
M2	23
M3	-5
M4	8

Patient Metabolic profile of a patient



Microbial composition



Using an adaptation of the PRMT method we can derive a **Microbial dictionary of biochemical/metabolic functions,** containing functional/metabolic information about all microbes in all individuals we collected data from (patient specific microbial composition and healthy individual microbial compositions).

We do not know yet how to derive the desired metabolic profile (reference estimated metabolome ...see next slide :)

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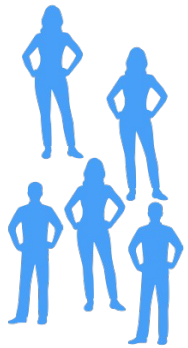
Larsen PE, Collart FR, Field D, Meyer F, Keegan KP, Henry CS, McGrath J Quinn J, Gilbert JA Predicted Relative Metabolomic Turnover (PRMT): determining metabolic turnover from a coastal marine metagenomic dataset. Microb Inform Exp. 2011 Jun 14;1(1):4. doi: 10.1186/2042-5783-1-4.
.

What do we need?A proper mathematical formulation for the problem

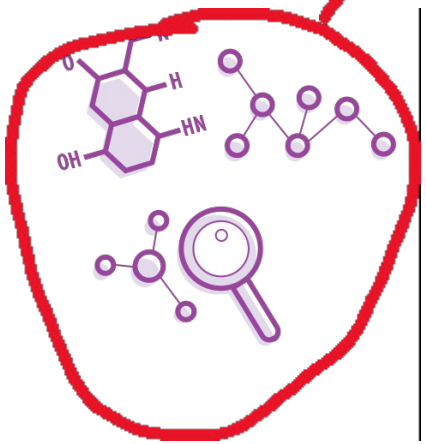
$$\hat{w} = \underset{w \in \mathbb{R}^{+P}}{argmin} \|Sw - d\|^2 \quad \text{such that} \quad \|w\|_1 \leq 100$$

Thanks to the matematical formulation we gave to the problem, we can derive the metabolic profile of both healthy and diseased individuals

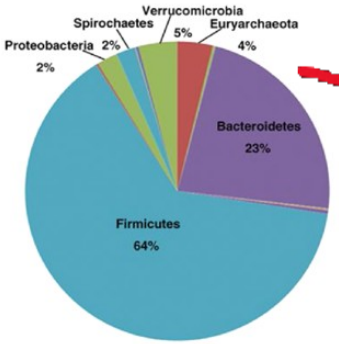
Healthy population



Healthy metabolic profile



Microbial composition



PRMT metabolites scores associated to microbes in our dictionary

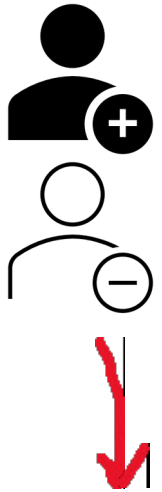
	Metabolity			
Druh mikrobu	M1	M2	M3	M4
Methanobrevibacter	2	0	10	5
Oscllibacter	5	0	3	0
Akkermansia	0	4	12	0
Treponema	1	6	0	0

X

A type of microbe	%
Methanobrevibacter	?
Oscllibacter	?
Akkermansia	?
Treponema	?

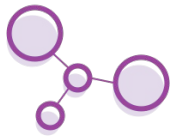
=

Metabolity	PRMT score
M1	50
M2	23
M3	-5
M4	8

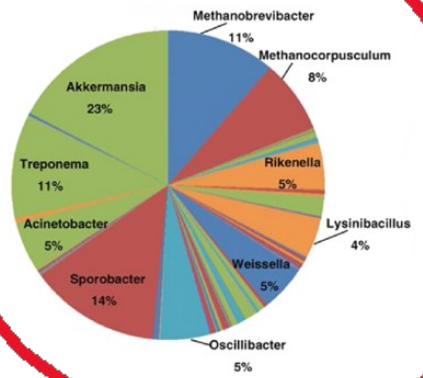


Patient

Metabolic profile of a patient



Microbial composition



S

Microbial dictionary of biochemical /metabolic functions

X

W

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…
…
…
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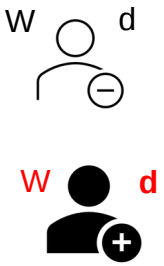
=

d

"C00084"=11.02
"C00473" =-3.05
"C05577"=-7.18
"C07490" =1.29
"C16551"=-1.70
"C16587"=3.01
"C16596"=4.47
"C00577"=10.00
"C00116" =-6.77
"C00441"=25.00
"C00191"=0.4
"C11402"=21.46
"C04517"=-87.46
-"C03752"=31.02
"C06192"=8.77
"C11418"=-750



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MiMetDec – a method of deconvolution of microbial profiles based on their metabolic potential

Principle: Basis pursuit functional approximation

What does it do?The tool takes an (estimated) metabolic profile, a library of microbial profiles and estimates th microbial composition that would lead to that d reference metabolic profile

The output of the procedure is the w^ or rebalanced microbial community.

	Metability			
A type of microbe	M1	M2	M3	M4
Methanobrevibacter	2	0	10	5
Oscllibacter	5	0	3	0
Akkermansia	0	4	12	0
Treponema	1	6	0	0

$$\hat{w} = \underset{w \in \mathbb{R}^{+p}}{argmin} \|Sw - d\|^2 \quad \text{such that} \quad \|w\|_1 \leq 100$$

A type of microbe	%
Methanobrevibacter	?
Oscllibacter	?

Metability	PRMT score
M1	50
M2	23

How can we use this method?

Find all microbial compositions capable of providing the same metabolic profile (phenotype).

To find out how to specifically modify the microbial composition of the environment (e.g. intestinal microbiome) to obtain the desired metabolic profile.

To find out which microbes are most important for a **certain type of metabolism**

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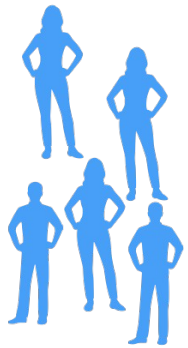
What do we need to explore microbes as bioterapheutics?

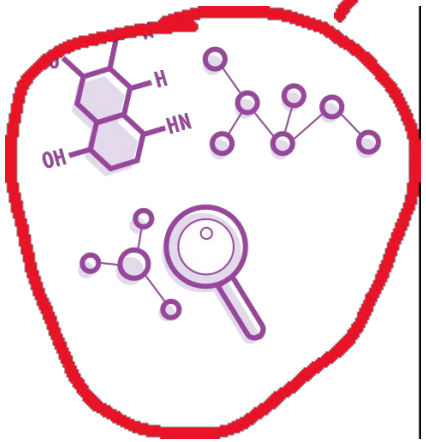
A method that estimates the microbial composition based on the desired metabolic profile

We have already derived the (reference) metabolic profile of healthy individuals...the closest for each patient...its **healthy prototype**

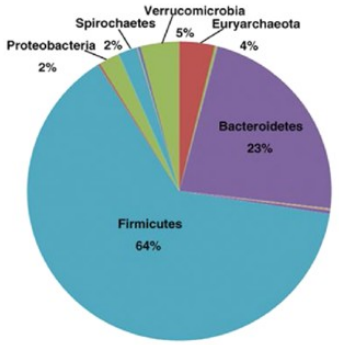
Healthy population



Healthy metabolic profile



Microbial composition



we can expand our intial dictionary of microbial metabolic functions

	Metabolity			
Druh mikrobu	M1	M2	M 3	M4
Methanobrevibacter	2	0	1 0	5
Oscllibacter	5	0	3	0
Akkermansia	0	4	1 2	0
Treponema	1	6	0	0
Lactobacillus	0	0	1	10

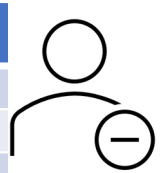
X

A type of microbe	%
Methanobrevibacter	?
Oscllibacter	?
Akkermansia	?
Treponema	?

≅

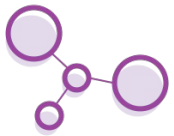
Metabolity	PRMT score
M1	50
M2	23
M3	-5
M4	8

d

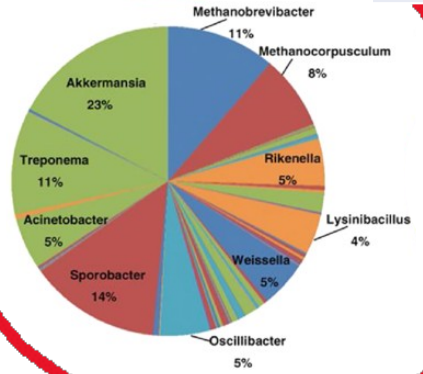


Patient

Metabolic profile of a patient



Microbial composition



We can explore in silico intervention with probiotics or putative biotherapeutucs!

What must be the microbial composition (the abundance of individual microbes of the patient) in order to achieve the metabolic profile of a healthy individual?

The tool will estimate for us the composition of the new therapeutic microbial community

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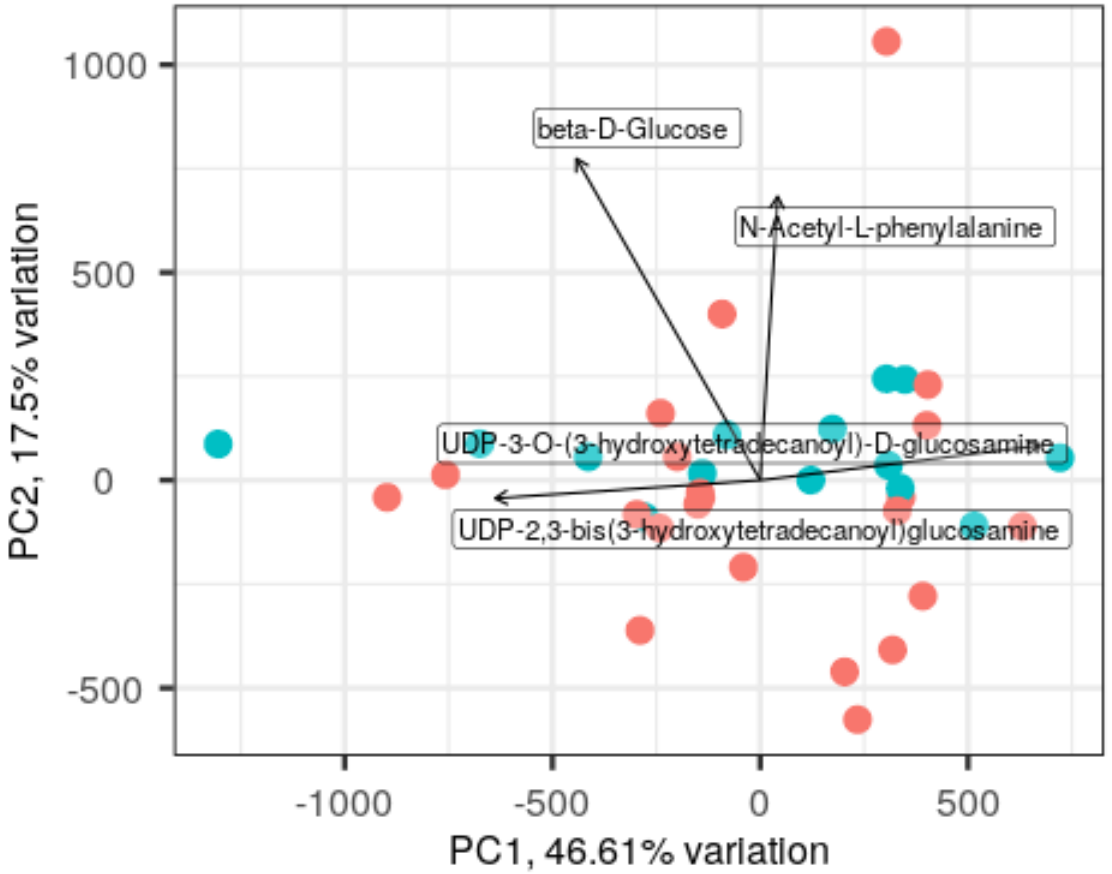
How does the tool work in a real example? - the gut microbiome in colorectal cancer

Wirbel et al, Nature. https://doi.org/10.1038/s41591-019-0406-6

We exploited already processed data(Wirbel's validation cohort:
22 patients with CRC, 16 healthy controls
Metagenome sequencing from fecal samples => species composition of bacteria=> our best guest strain resolution level

Methodology:
1. Estimation of the metabolic profile of hypotized strains
2. Finding a bacterial healthy prototype (11 found)
3. Estimation of changes in the patient's microbial profile based on a healthy prototype (d^=Sw^)

The estimated metabolic profiles of patients and controls suggest significantly different profiles even in healthy individuals



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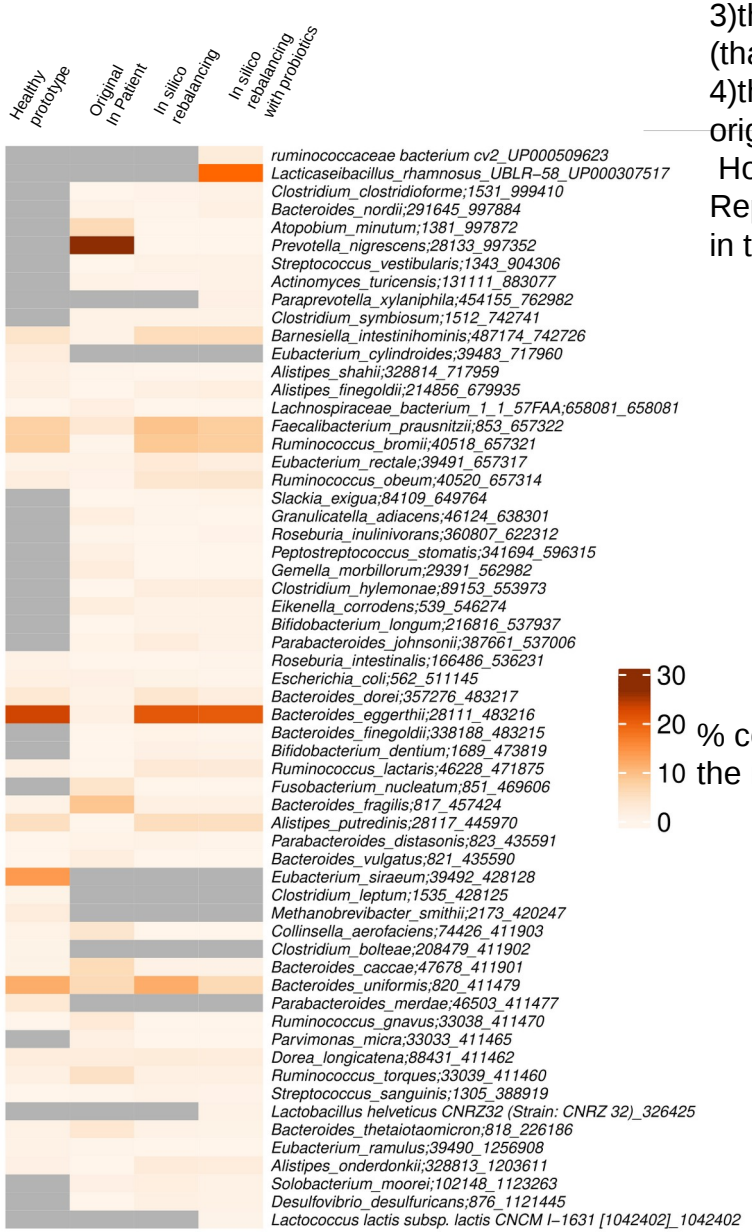
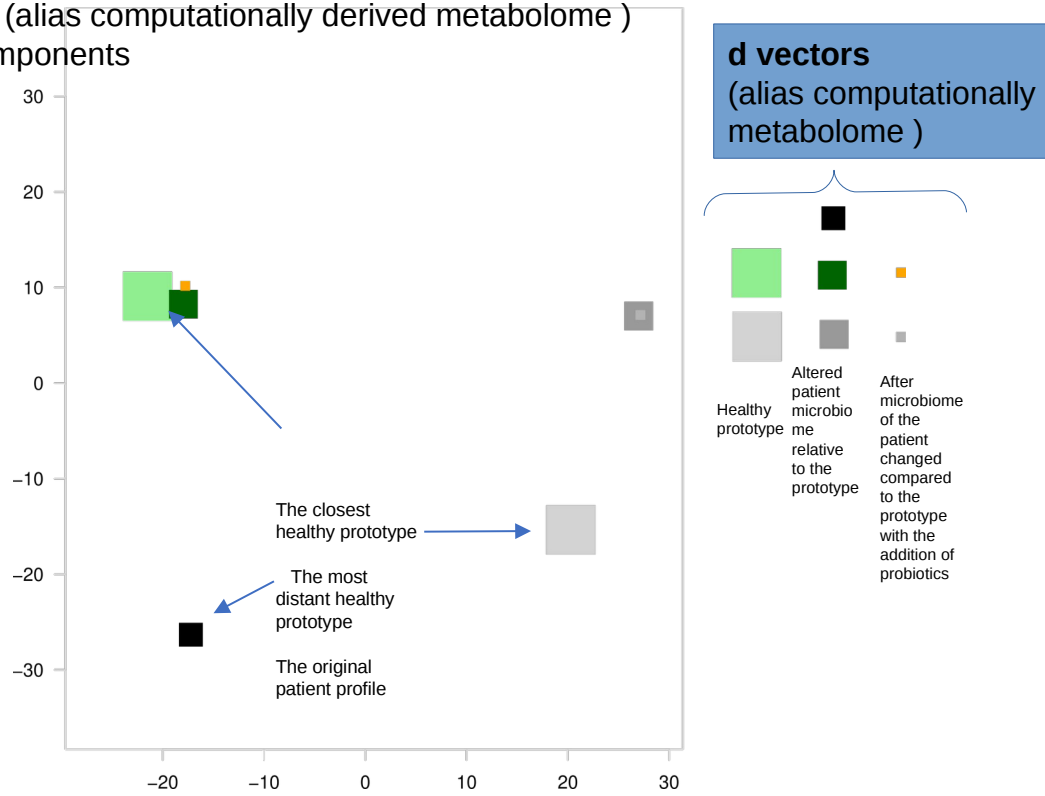
Example of one patient

In silico experiment,where we have our estimated metabolomes:
1)the original computationally derived metabolome of the patient (black square)
2)the computationally derived metabolome of the healthy prototype (green light)
3)the computationally derived metabolome of the rebalanced microbial community (that is the output of our method) (dark green)
4)the computationally derived metabolome after a bioterapeutics based intervention (feeding the procedure with original patient flora + bioterapeutical community)(orange)

How did the patient approximate the metabolic profile of the healthy prototype in the *in silico experiment*?
Representing the **d vectors** (alias computationally derived metabolome) in the space of Principal Components



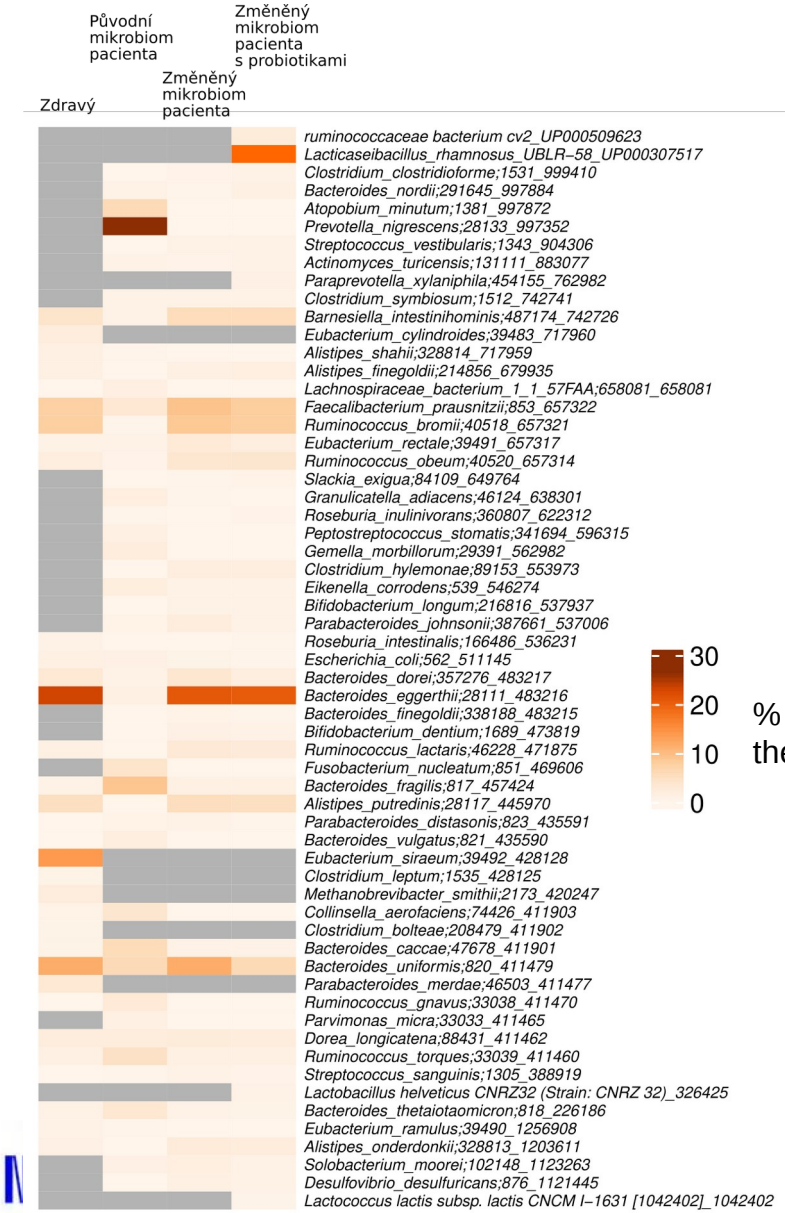


d vectors
(alias computationally derived metabolome)

$$\hat{w} = \underset{w \in \mathbf{R}^{+p}}{argmin} \ \|Sw - d\|^2 \quad \text{such that} \quad \|w\|_1 \leq 100$$

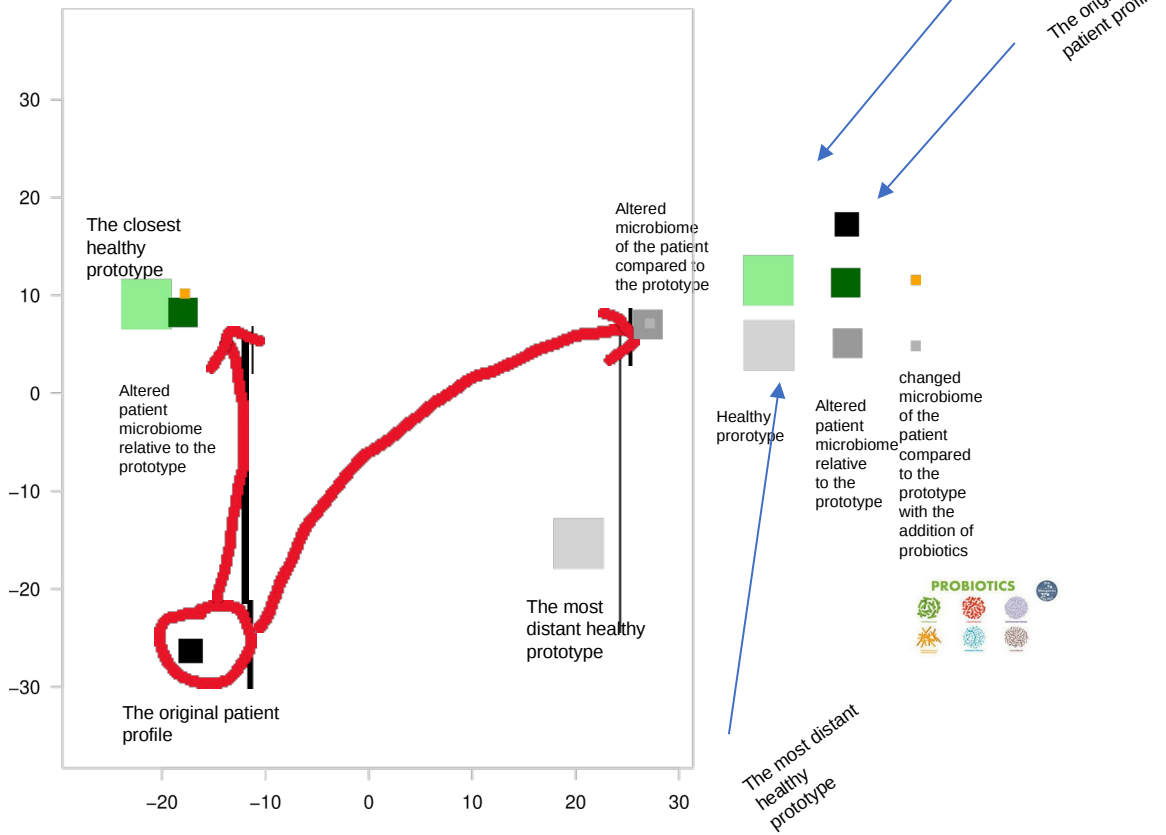
(d^=Sw^)

Example of one patient



% composition of the microbiome

How did the patient approximate the metabolic profile of the healthy prototype?



What's next?

- implementation of the method into a package in R/Bioconductor
- incorporating the effects of XENOBIOTICS
- incorporating the HOST's metabolism

External collaborator

Daniela de Canditiis
Italian National Research Council | CNR · Institute for Applied Mathematics "Mauro Picone" IAC

Thanks for your attention :)

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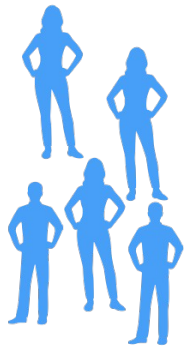
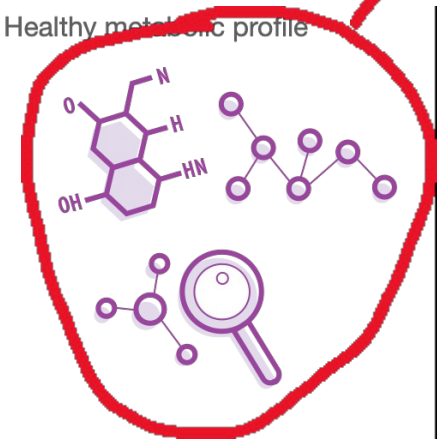
What do we need?

A method that estimates the microbial composition based on the desired metabolic profile

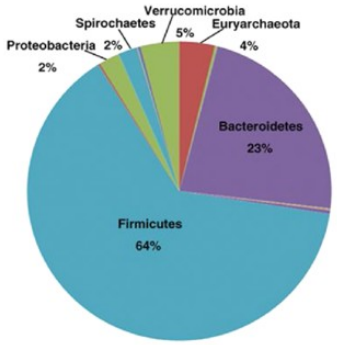
Healthy population



Healthy metabolic profile

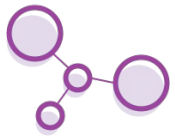


Microbial composition

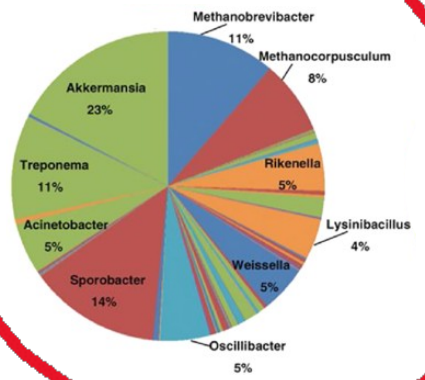


Patient

Metabolic profile of a patient



Microbial composition



What is the metabolic profile of a healthy individual?

Thanks to our clever experimental design we have also collected data from healthy individuals...and by interrogating the appropriate database....

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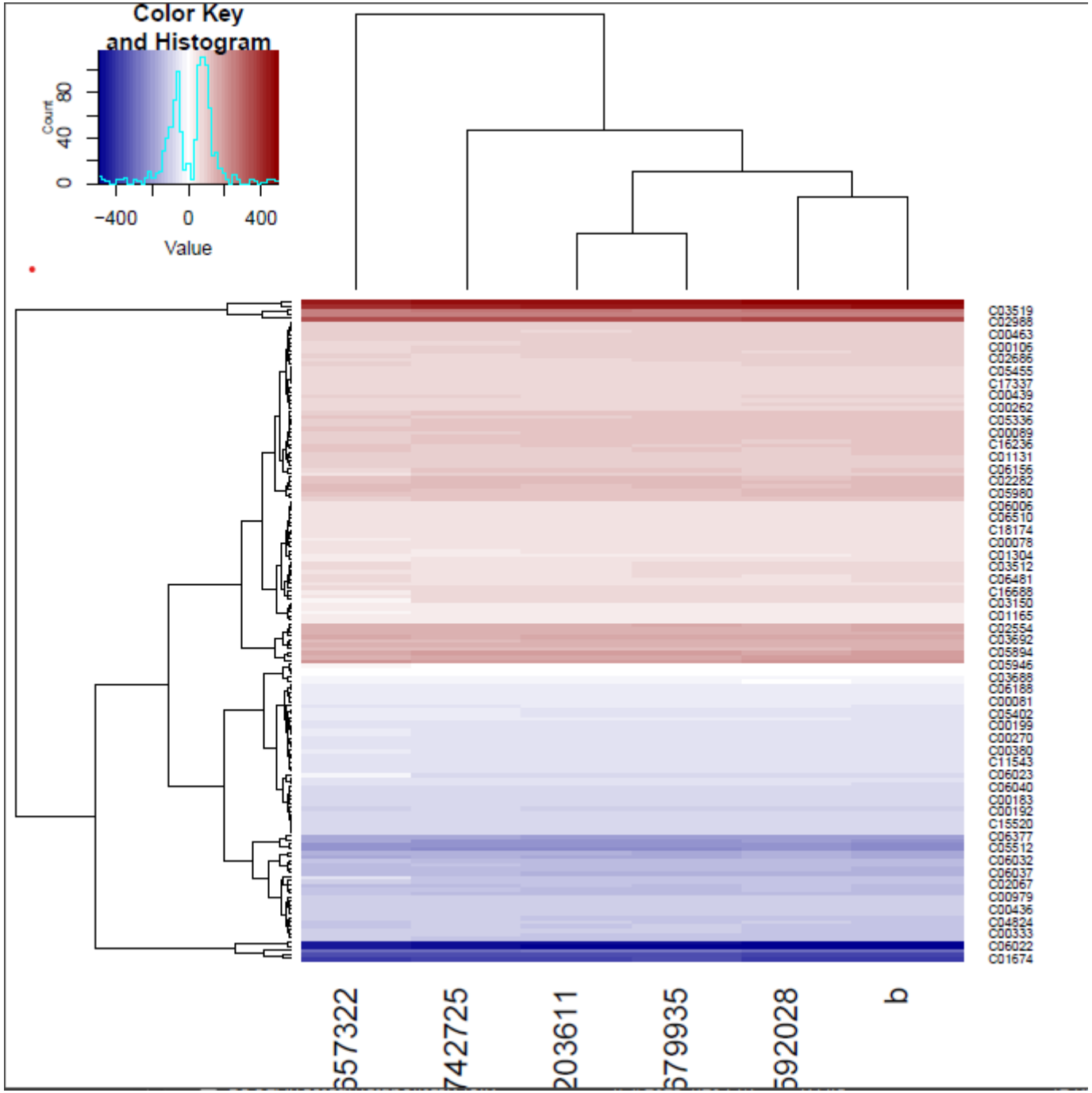
- to identify association between a microbe and a specific metabolite produced or consumed by this microbe, we can perform a kind of leave one out procedure
- filtering the result is of fundamental importance
  -computations are quite efficient



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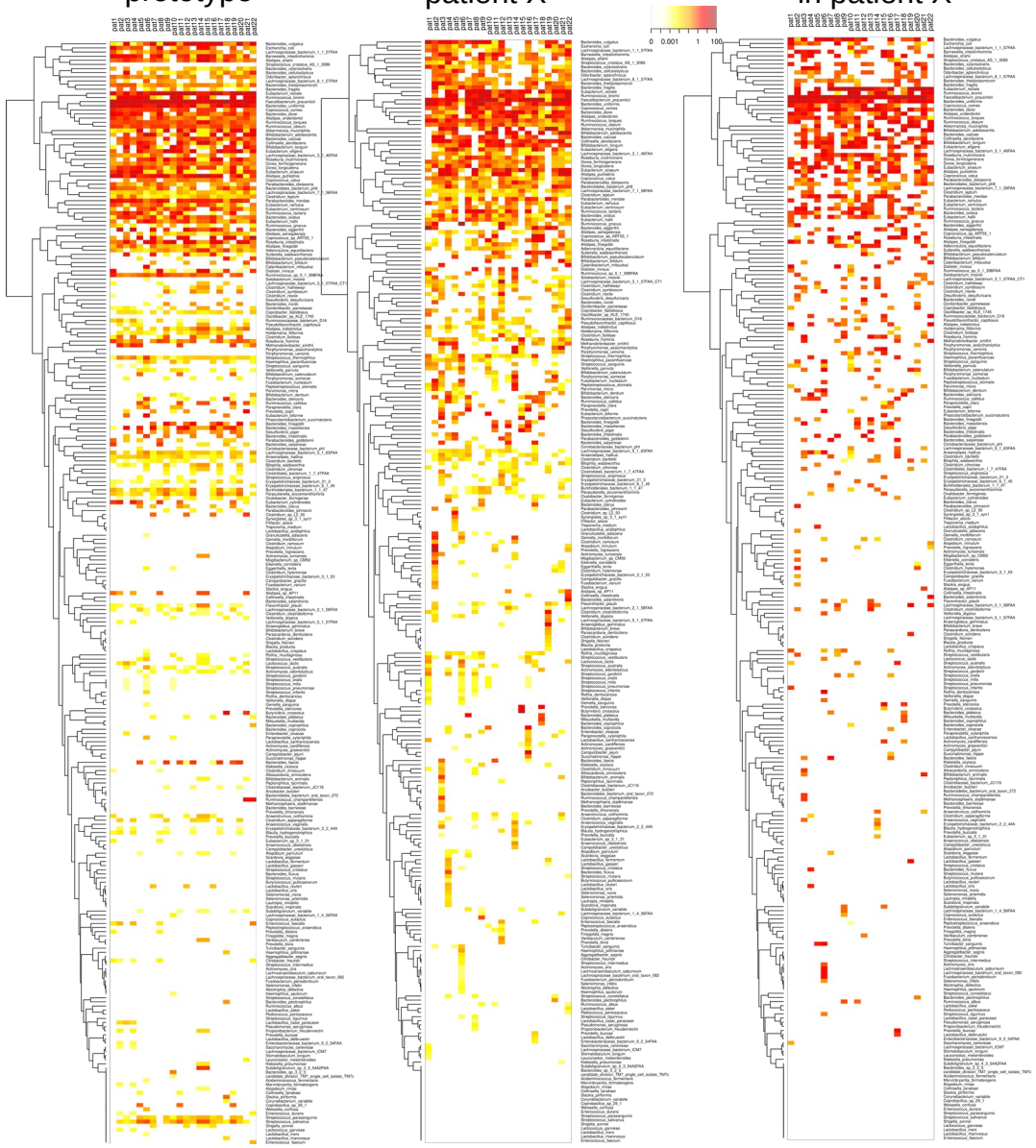
How does the tool work in a real example - the gut microbiome in colorectal cancer – estimated relative abundances of strains in *in silico* adjusted profiles

Healthy prototype

Original profile patient X

Adjusted profile in patient X



%composition of the microbiome

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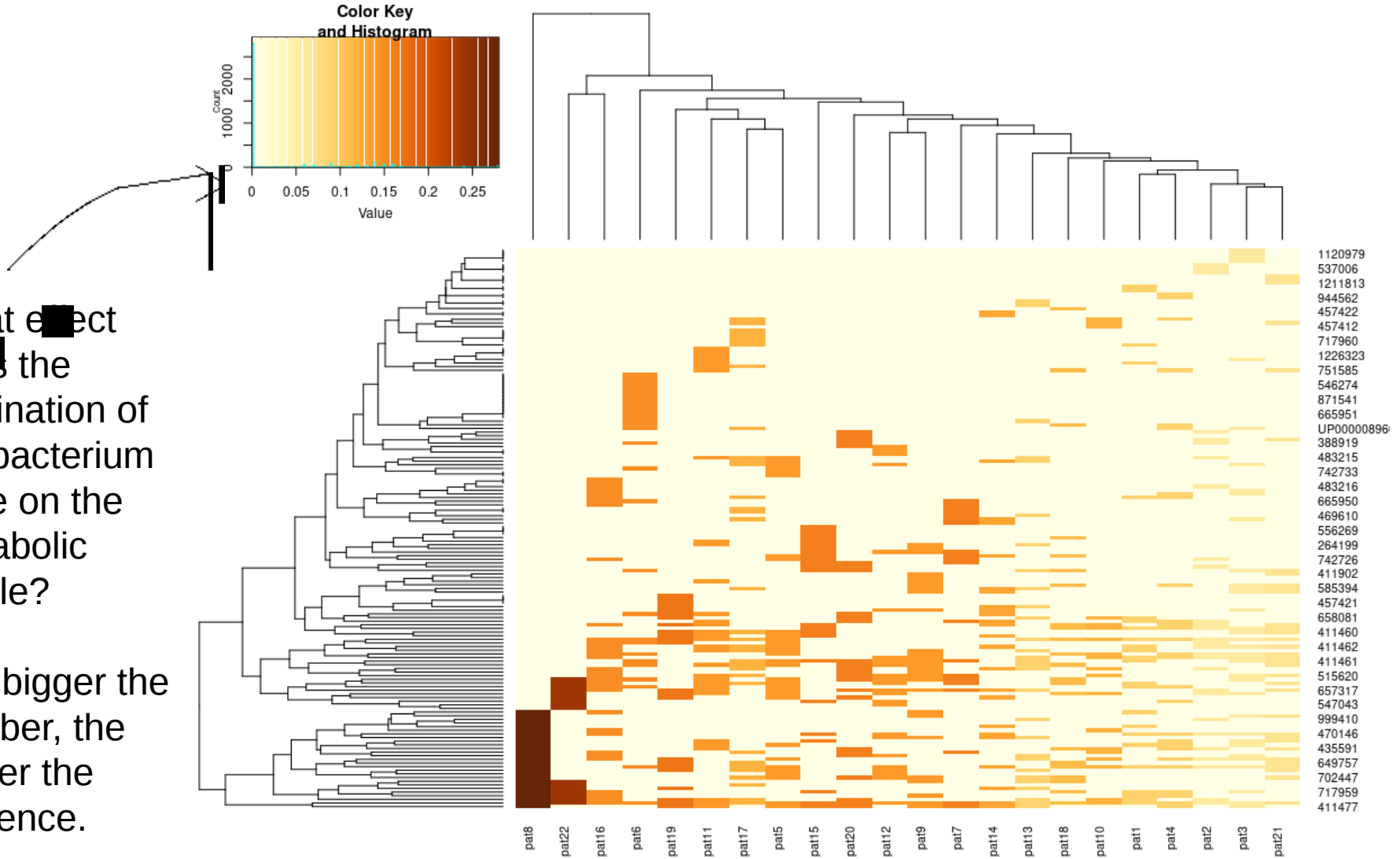
Which bacteria changed most often and their elimination caused the biggest problems?



What effect does the elimination of the bacterium have on the metabolic profile?

The bigger the number, the bigger the influence.

Microbes (taxon ids)

Patients

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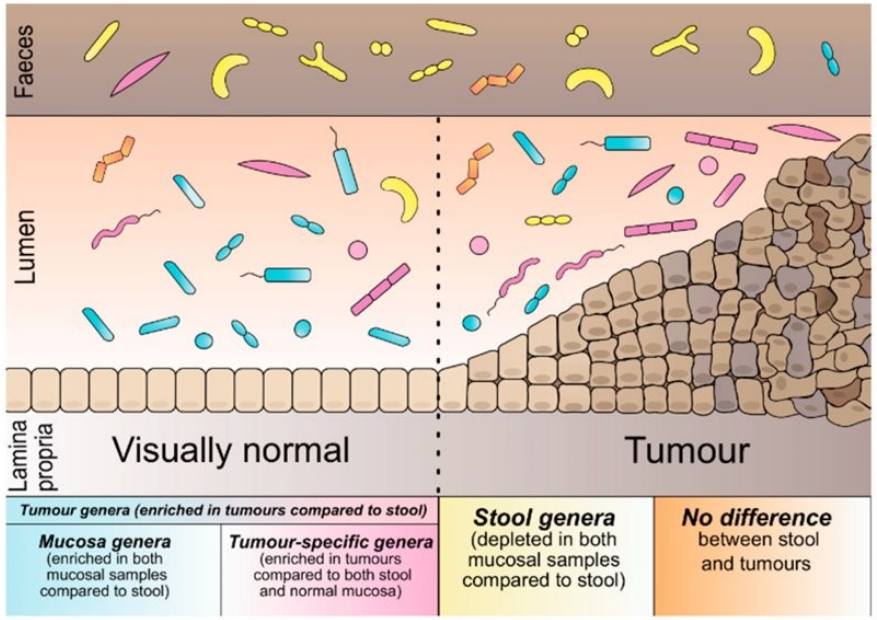
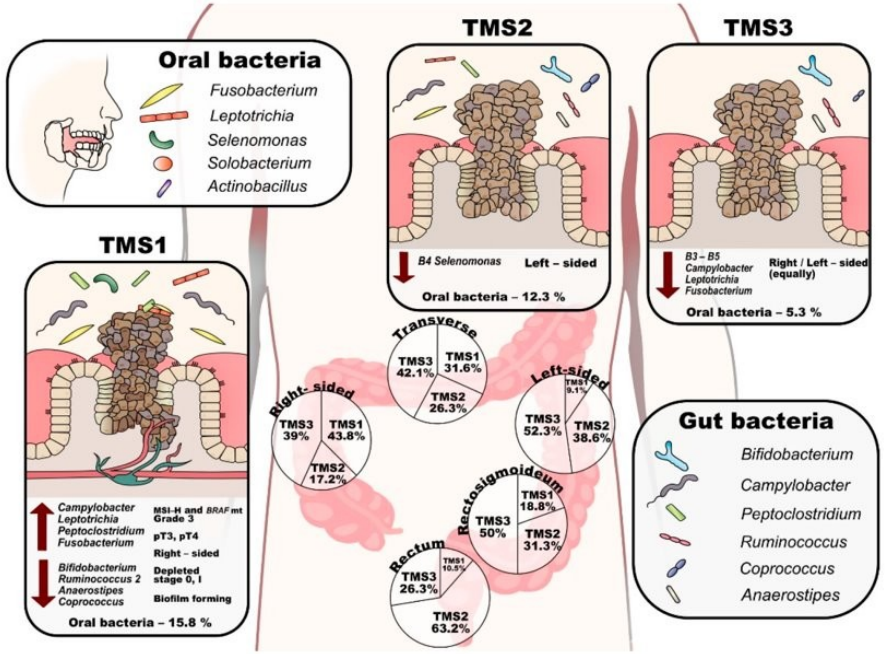
Comparison of the microbiome in the stool and in the tumor



The tumor microbiome is different from the microbiome of healthy tissue and stool
It is enriched with potential oral pathogens



TMS – tumor microbiome subtypes

Tumors are divided into three basic groups according to their microbiome

Zwinsova et al., 2021, Cancers 10.3390/cancers13194799

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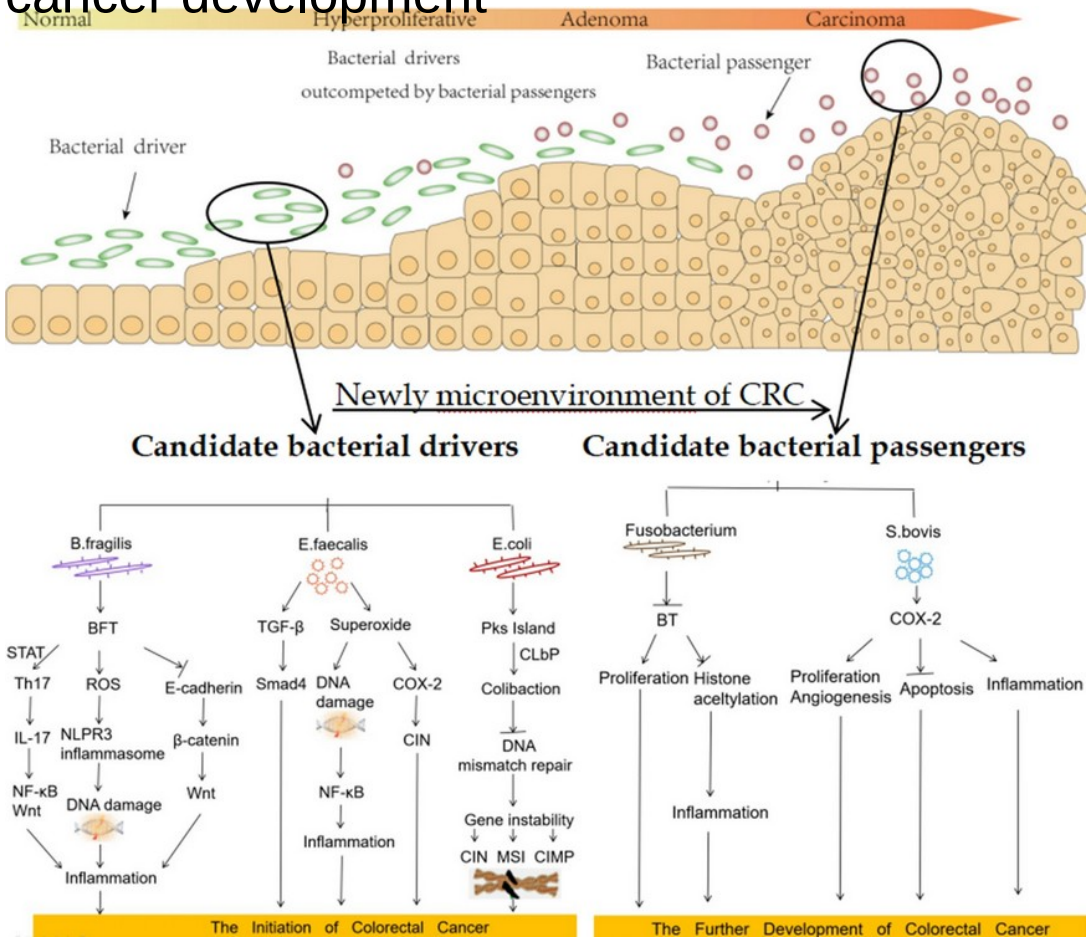
How does the tool work in a real example? - the gut microbiome in colorectal cancer

Colorectal cancer - a very heterogeneous disease.

Bacteria affect the tumor
- positively: they expose the tumor to the immune response -
- negatively - they worsen the prognosis, hide the tumor from the immune system, influence the response to therapy, cause additional mutations with their genotoxic products

*"Bacterial passengers of CRC are defined as **gut bacteria that are relatively poor colonizers of a healthy intestinal tract but have a competitive advantage in the tumour microenvironment**, allowing them to outcompete bacterial drivers of CRC"

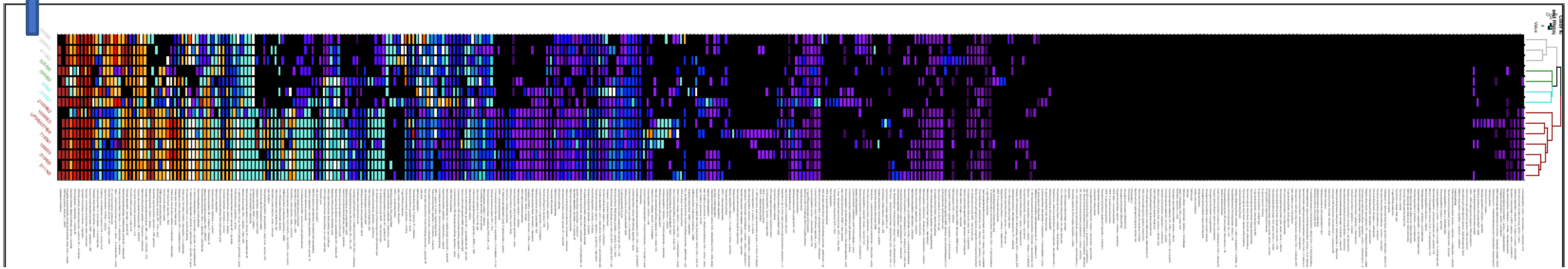
Bacterial "driver-passenger" model* of colorectal cancer development



https://link.springer.com/article/10.1007/s12094-021-02738-y/figures/3

IF WE WANT …

Taxon ids of microbilal strains selected by the tool when an ad hoc reference metabolic profile has been provided as constraint of the problem



```
> sort(wHat_unknown_metabTasks[which(wHat_unknown_metabTasks != 0)])
      29466      310297      658086      556269      999412     1006003      411902      525338      716541 UP000037484      511145     2100482
  0.2028664   0.2583832   0.3509318   1.9129620   2.3407463   2.7972778   2.9709230   4.7247821   7.3806503   8.3724402  17.2127286  21.1114636
     198215
 30.3638442
```

- to identify a small community of microbial strains having functional similarity with a microbe leader that we already know as probiotics, we can build a reference metabolome (d) ad hoc to pursue this goal
- using E Coli Niesle as microbe leader, we identified other 13 microbes constituting together a putative therapeutical cocktail of microbes
  - the tool estimates the relative abundances of each member of the therapeutical cocktail

Description of modules

```
[1] "Proline biosynthesis, glutamate => proline"
[2] "Ornithine biosynthesis, glutamate => ornithine"
[3] "Urea cycle"
[4] "Creatine pathway"
[5] "Ornithine biosynthesis, mediated by LysW, glutamate => ornithine"
[6] "Arginine biosynthesis, ornithine => arginine"
[7] "Arginine biosynthesis, glutamate => acetylcitrulline => arginine"
```

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