Microbioma intestinale e carcinogenesi
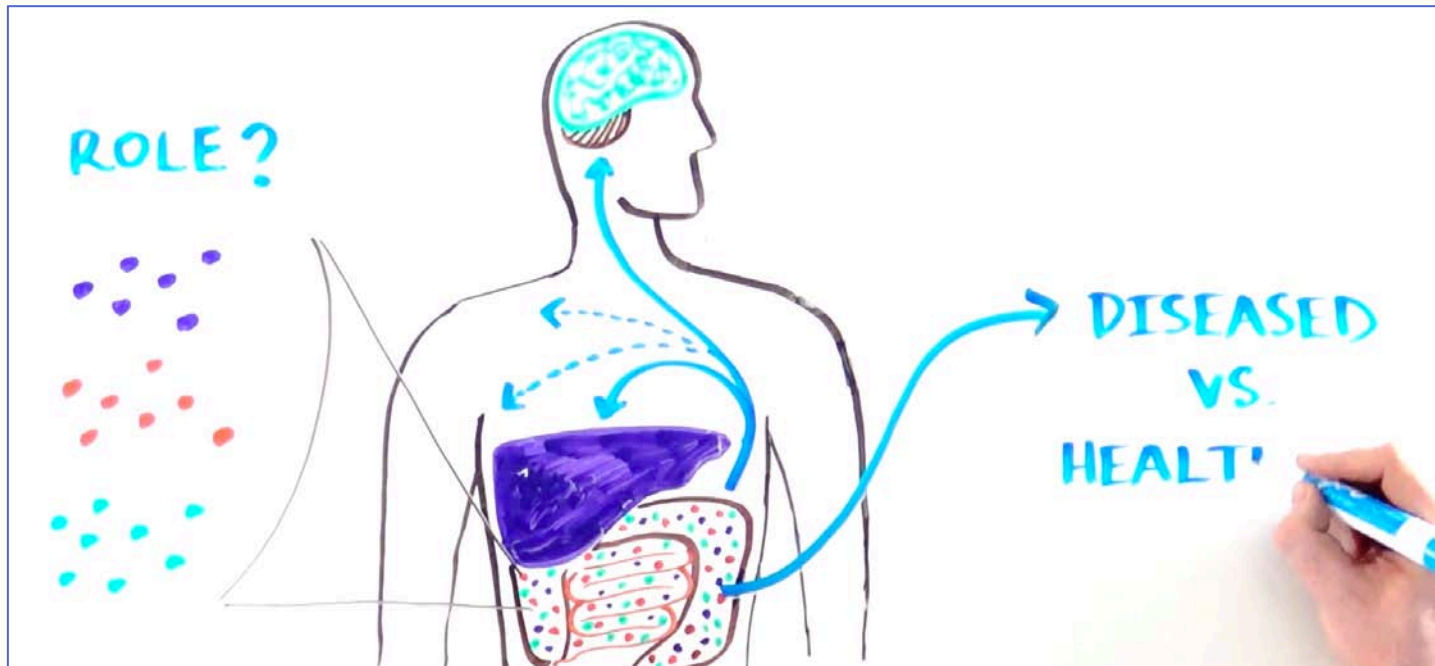
Maria Rosalia Pasca

What's the Microbiome?

> *"…the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease"*
>
> **Joshua Lederberg**
> **The Scientist 15[7]:8, Apr. 2, 2001**

- These microbes are **not harmful** to us;
- they are **essential for maintaining health**;
- they produce some **vitamins** that we do not have the genes to make;
- break down our **food** to extract **nutrients** we need to survive;
- teach our **immune system** how to recognize dangerous invaders.

- Microbial communities of Microbiota:

    Bacteria,
    Archea ;
    Eukarya → Mycobiome;
    Virus → Virome

100 trillions
Number of microbes in our gut

The **intestine** is a preferred site – over **70%** of all bacteria are found in the colon.
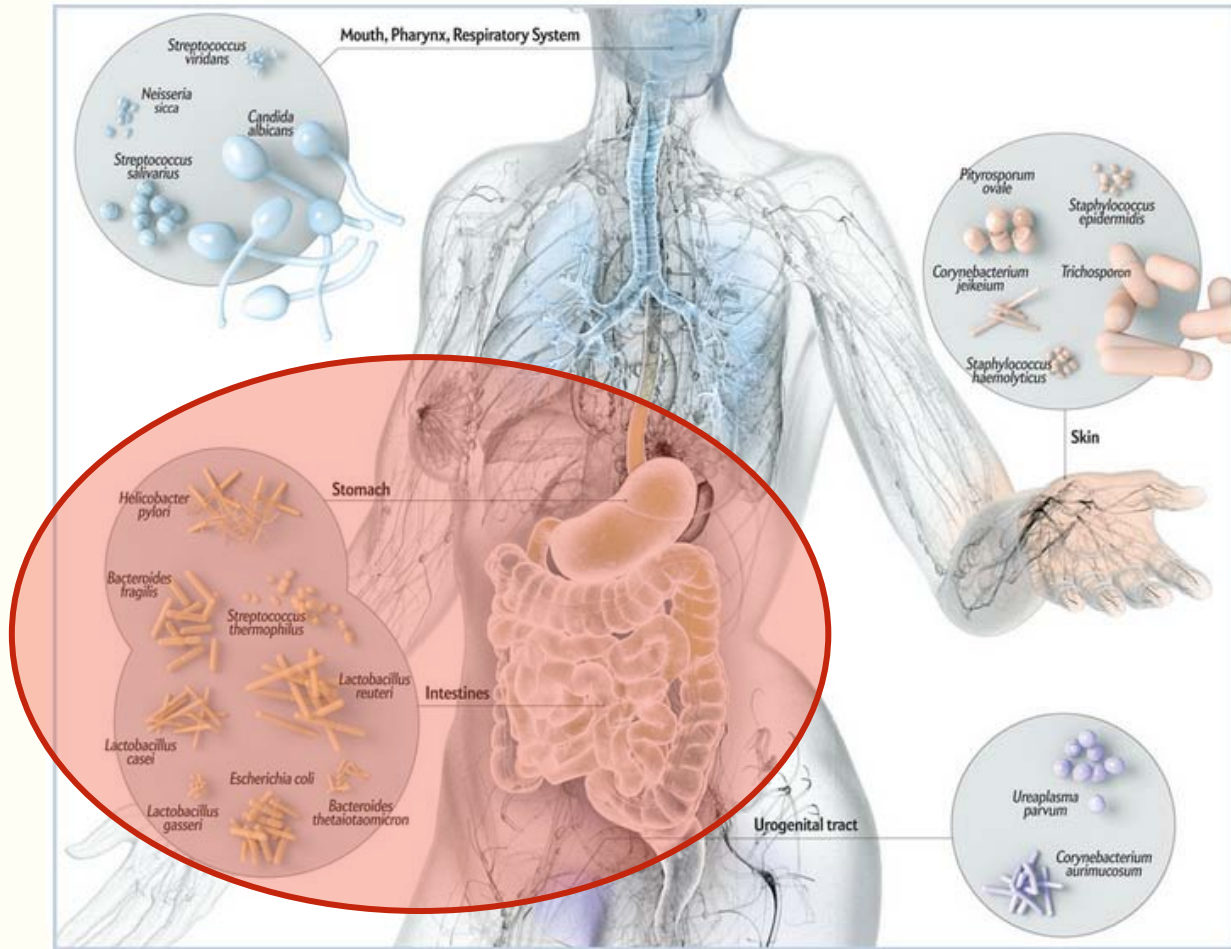
90%
of cells in and on our body are **bacterial cells**.

10:1
Ratio of nonhuman cells to human cells in our body

22.000
Number of human genes in the human gene catalog

>1.000.000
Number of genes in our microbiome

Composition of gut microbiota

- There is wide spatial and temporal variation within the same intestine and between **individuals**, **ages**, **cultures**, **diet** (e.g.: vegetarian, vegan, etc.) and **sexes**.

- Although 4 phyla (**Firmicutes**, **Bacteroidetes**, **Actinobacteria**, and **Proteobacteria**) dominate the human microbiota, the makeup of a person's microbiota can be considered as **individual fingerprint**.
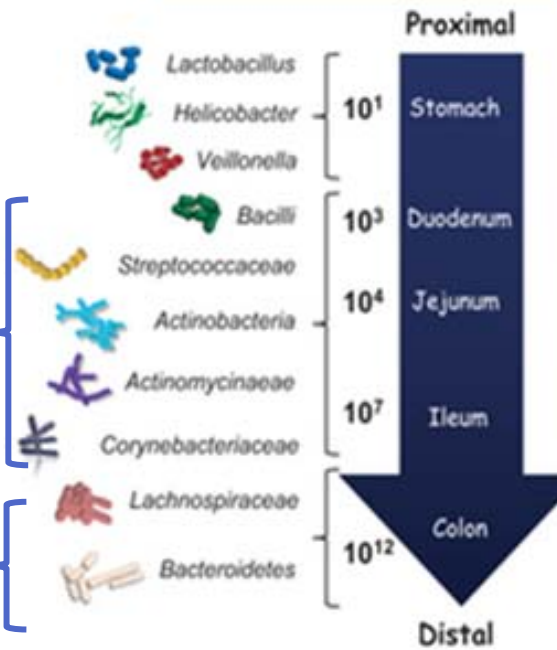
Phyla	Genera
Bacteria	
Firmicutes (30-50%)	*Ruminococcus*
	Clostridium
	Peptostreptococcus
	Lactobacillus
	Enterococcus
Bacteroidetes (20-40%)	*Bacteroidetes*
Actinobacteria (3-15%)	*Bifidobacterium*
Proteobacteria (1-10%)	*Desulfovibrio*
	Escherichia
	Helicobacter
Verrucomicrobia (0.1%)	
Archea	
Euryarchaeota	*Methanobrevibacter smithii*

Longitudinal
Bacteria increase in number and composition changes from proximal to distal GI tract.

Gastrointestinal tract

Proximal

| Lactobacillus |
| Helicobacter | 10^1 | Stomach |
| Veillonella |

| Bacilli | 10^3 | Duodenum |
| Streptococcaceae |
| Actinobacteria | 10^4 | Jejunum |
| Actinomycinaeae |
| Corynebacteriaceae | 10^7 | Ileum |

| Lachnospiraceae |
| Bacteroidetes | 10^{12} | Colon |

Distal

- **Bacteroidetes + Actinobacteria (50%)**
- **Firmicutes (40%)**

Bacteroidetes + Actinobacteria (90%)

Latitudinal
Bacterial composition also differs between lumen, mucus, and attached to epithelium.

Clostridium
Lactobacillus
Enterococcus

Bacteroides   Ruminococcus
Bifidobacterium   Clostridium
Streptococcus   Lactobacillus
Enterobacteriacea   Enterococcus

Epithelium | Epithelial surface   Mucus layer   Intestinal lumen | Feces

Interact with host's **immune system**.

Interact with **food** and digested **metabolites**.

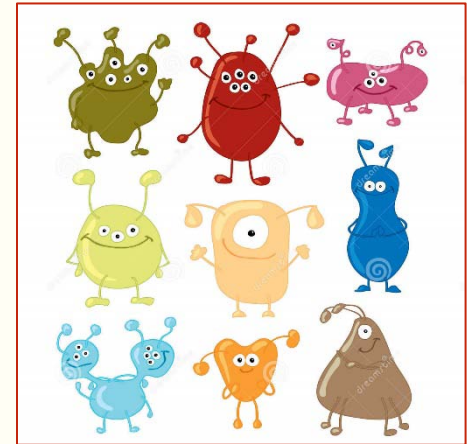Bakhtiar *et al*. 2013. FEMS Microbiol Lett.; Gagnière *et al*., 2016. Wo

From microbiome characterization to health impact

• **Modification in the bacterial composition of microbiome** could be alter our state of health.

Healthy associated typical profile

EUBIOSIS
- Bacterial groups are protective against pathogens;
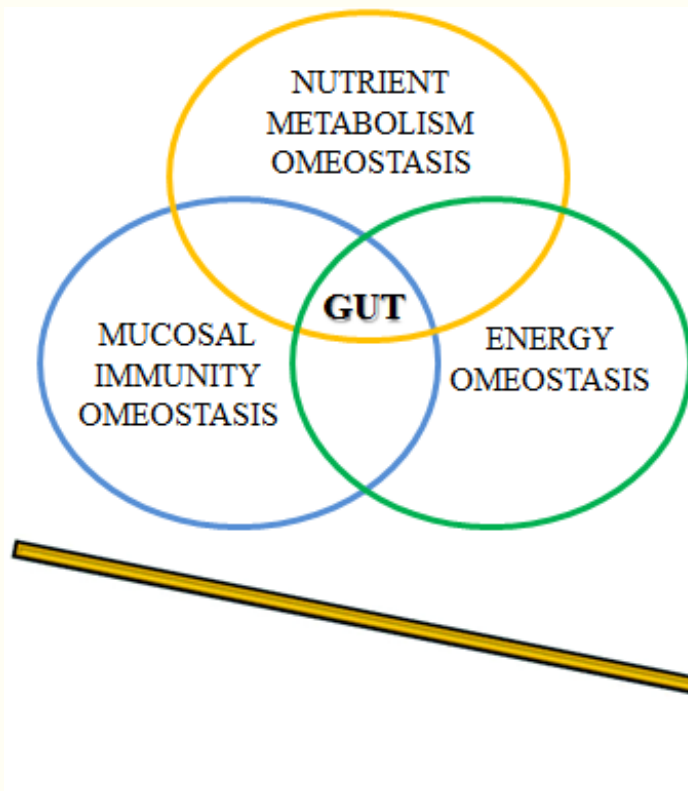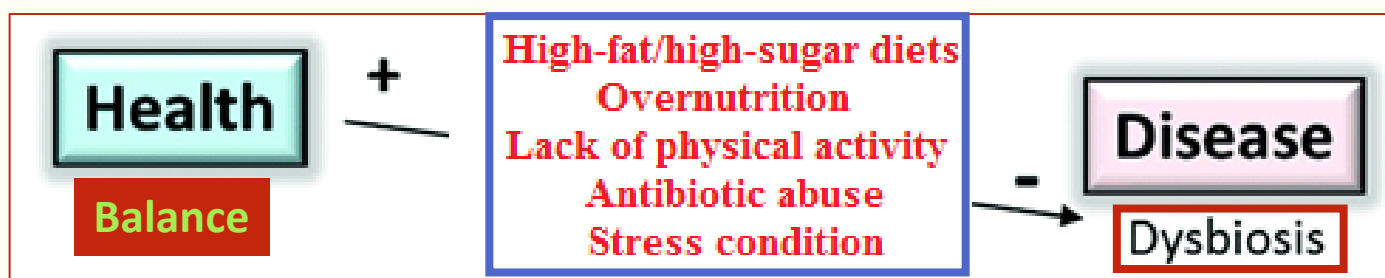- The intestinal microbial ecosystem is in balance.

Disease associated typical profile

DYSBIOSIS
- Bacterial groups are associated to several pathological conditions;
- There are qualitative and quantitative changes in the intestinal flora.

Health + Balance

High-fat/high-sugar diets
Overnutrition
Lack of physical activity
Antibiotic abuse
Stress condition

Disease - Dysbiosis

NUTRIENT METABOLISM OMEOSTASIS

MUCOSAL IMMUNITY OMEOSTASIS

GUT

ENERGY OMEOSTASIS

Dysbiosis is the possible cause of intestinal, metabolic and autoimmune diseases.

Intestinal:
- **IBD** (inflammatory bowel disease)
  - Crohn's disease (CD)
  - Ulcerative colitis (UC)
- **IBS** (irritable bowel syndrome)
- **Coeliac disease**
- **CRC** (colorectal cancer)
- **CDI** (*C. difficile* infection)

Extra-intestinal:
- **Obesity**
- **Type II diabetes**
- **Metabolic syndrome**
  - ↓ butyrate-producers
  - ↑ Opportunistic pathogens
  - ↑ Oxidative stress
- **Lupus erythematosus**
- **Allergy / Asthma**
- **Gut-brain axis disorders** (autism spectrum, Parkinson's, disorders of mood and chronic pain)

Treatment of dysbiosis → Reduction of the risk of the disease

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Colorectal Cancer (CRC) and microbiome dysbiosis

- **Toxins**
- **Virulence factors**
- **Metabolites**
- **ROS and NOS production**

- **Induction of DNA damage,**
- **genome instability,**
- **inflammation;**
- **interferences of apoptosis and cellular cycle;**
- **alterations of cellular components.**

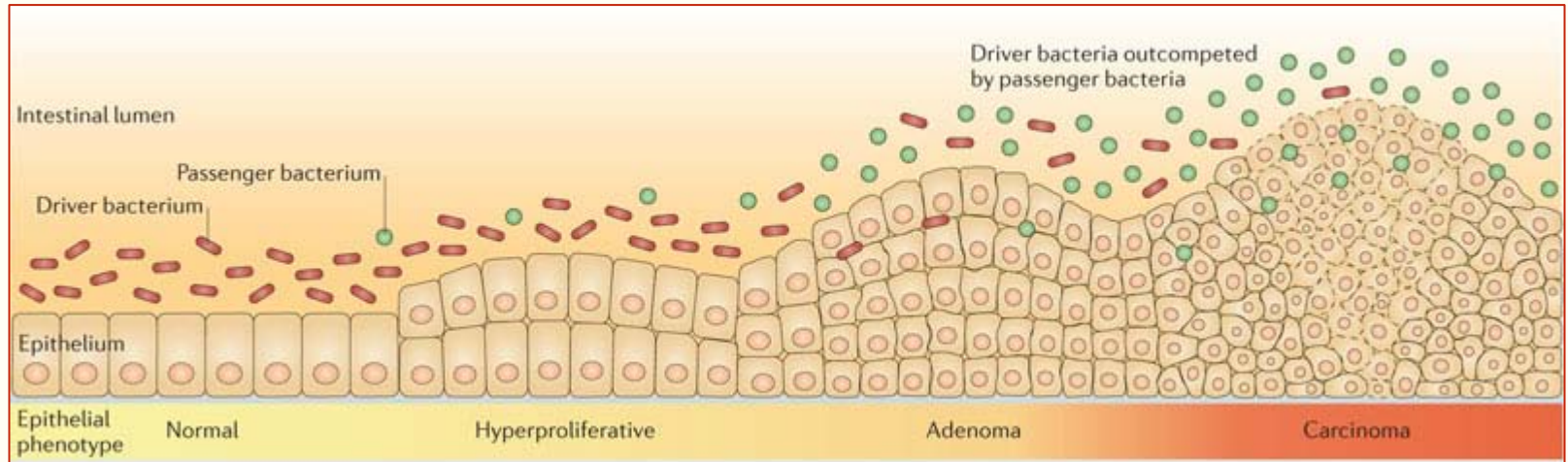Gagnière et al., 2016 *World J Gastroenterol*
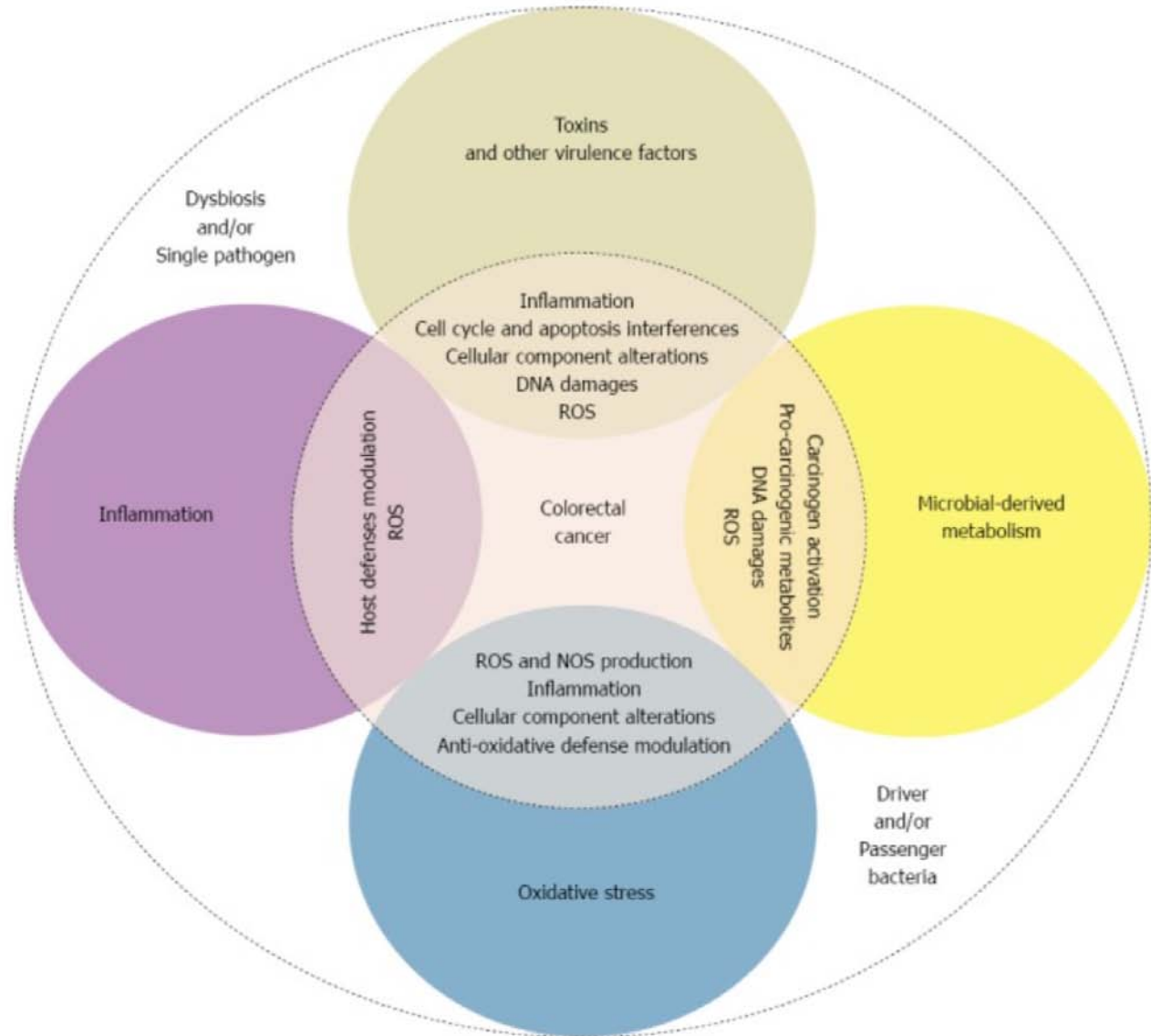
Putative bacterial species involved in colorectal carcinogenesis

Table 1 Summary of 16S rRNA sequencing and qPCR analyses of colonic microbiota variations in colorectal cancer

Variation in CRC	Phyla	Genus/species		
		Enterococcus Faecalis		
		Porphyromonas/Escherichia/Shigella Enteroco...		
		Streptococcus/Peptostreptococcus		
		Bacteroides fragilis		
		Bacteroides/Prevotella		
		Peptostreptococcus/Mogibacterium		
		Anaerococcus/Slakia/Paraprevotella		
		Anaerotruncus/Collinsella/Desulfovibric		
		Eubacterium/Porphyromonas		
		Atopobium/Porphyromonas		
		Fusobacterium		
		Fusobacterium/Bacteroides		
		Bacteroides/Fusobacterium		
		Alistipes/Escherichia/Parvimonas/Biloph...		
		Faecalibacterium prausnitsii		
		Bacteroides vulgatus/Bacteroides uniform...		
		Roseburia/Butyrate-producing bacteria		
		Faecalibacterium prausnitsii/Roseburia		
↓	Bacteroidetes			
↓		Ruminococcus		
↓	Firmicutes (clostridia)	Ruminococcus/Bifidobacterium/Streptococcus	46 CRC/63 C	[214]

- Increase of **Escherichia coli (phylogroups: B2 and D)** in CRC (in particular III and IV stage) → prognostic factor?
- B2 *E. coli* toxin (**colibactin**) interferes with **cell eukaryotic cycle** and induces **DNA damage** and **genomic instability** (in mouse model).
- D *E. coli* could be potentially pathogenic *via* downregulation of **DNA mismatch repair** system.

- Increase of **Enterotoxigenic Bacteroides fragilis** in CRC;
- It was demonstrated *in vitro* and in murine model that **BFT toxin** increased **cell proliferation** and **DNA damage**.

- Increase of **Enterococcus faecalis** in CRC;
- It could produce pro-oxidative reactive oxygen species (**ROS**).

- Increase of **Fusobacterium nucleatum** in adenomas and CRC (> 80%) → CRC promotion.
- This effect may be mediated by **FadA** adhesion and activates the **Wnt-β-catenin pathway** (in mouse model).

Sears and Garrett. 2014. Cell Host Micr; Gagnière *et al*. 2016 .*World J Gastroenterol.*

Driver and Passenger Bacteria

- **Driver bacteria** → iperproliferation and neoplastic transformation
- **Passenger bacteria** → last neoplastic transformation

Specific microbial associations
can be potentially used as biomarkers during neoplastic progression

Sears and Garrett. 2014. *Cell Host Micr.*; Tjalsma *et al*. 2012 *Nature Rev. Microbiol.*

Studying microbiome:
16S rRNA gene sequencing

- First step is to obtain **total bacterial DNA**.
- DNA from all bacteria present in a sample (**faeces**, **biopsies**) need to be recovered.
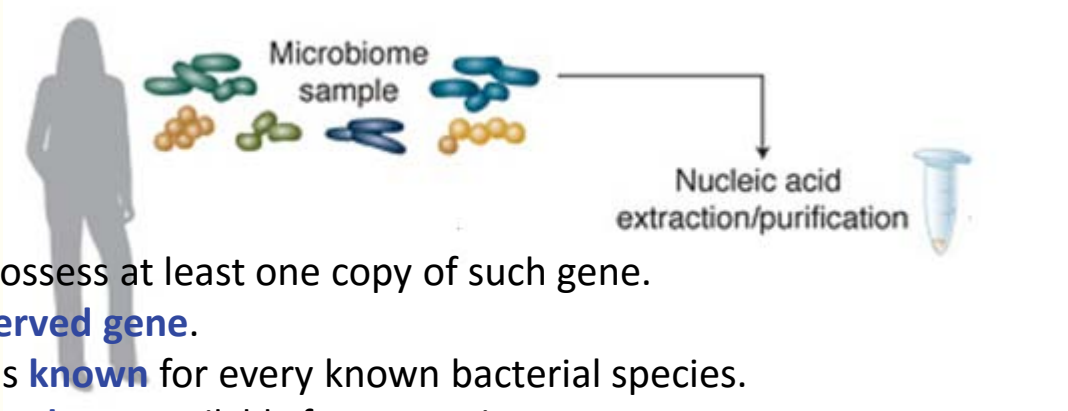
- All the organisms possess at least one copy of such gene.
- **Essential and conserved gene**.
- The 16S sequence is **known** for every known bacterial species.
  - **Large public databases** available for comparison.
- It is characterized by **9 high conserved regions** interspersed with **9 hypervariable regions** (**V1-V9**).
- **Variable sequence** can be thought of as a molecular "**fingerprint**".
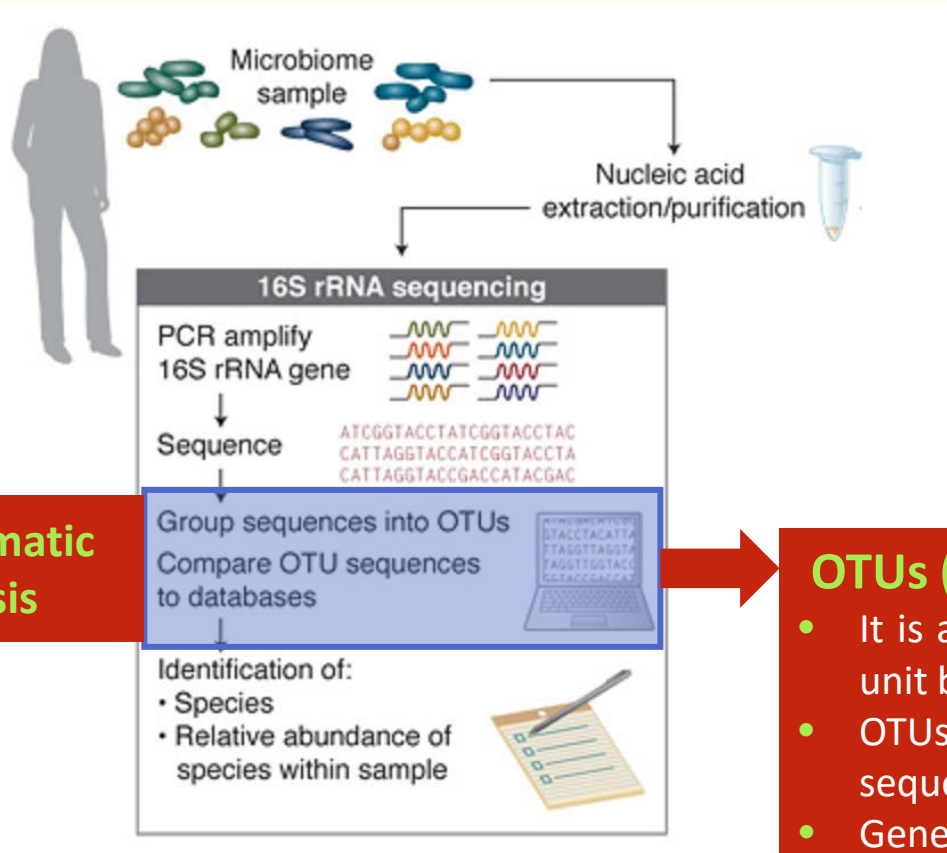 - Can be used to identify bacterial genera and species.

16S rRNA gene

The best phylogenetic marker for microbiota characterization
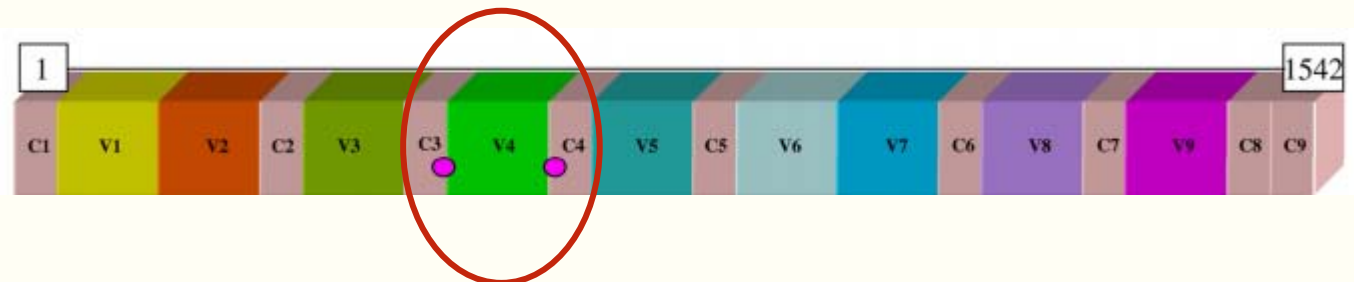
V4 16S gene libraries and **Illumina sequencing**

Modified from Petrosino et al., 2009 *Clin. Chem.*

NEXT GENERATION SEQUENCING

Microbiome sample

Nucleic acid extraction/purification

16S rRNA sequencing

PCR amplify 16S rRNA gene

Sequence

ATCGGTACCTATCGGTACCTAC
CATTAGGTACCATCGGTACCTA
CATTAGGTACCGACCATACGAC

Group sequences into OTUs

Compare OTU sequences to databases

Identification of:
- Species
- Relative abundance of species within sample

Bioinformatic analysis

OTUs (Operational Taxonomic unit)
- It is an arbitrary definition of a taxonomic unit based on sequence divergence.
- OTUs are number of clusters of similar sequences (**reads**).
- Generally, when 16S sequences are clustered at **97% identity ~ species**.

Modified from Stewart and Yigit, New England Biolabs, Inc.

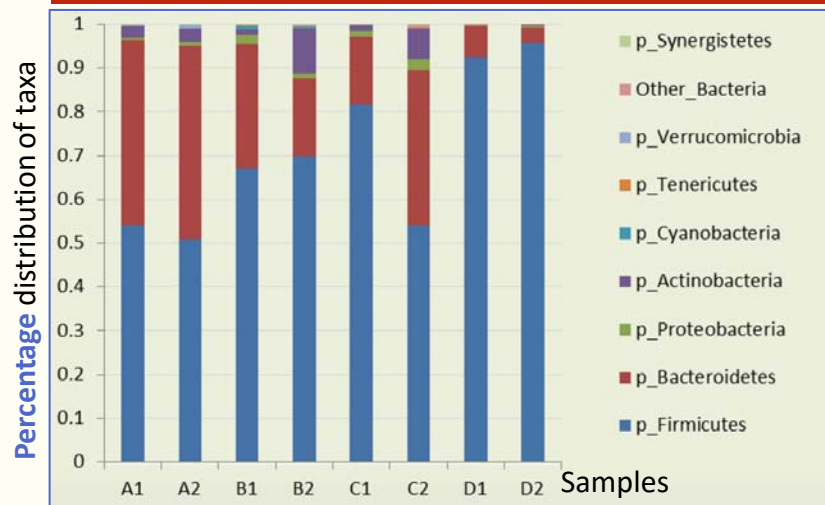| CancerType	Healthy	CRC	CRC	Healthy	CRC
Samples					
#OTU ID	SC255	SC131	SC276	SC104	SC250
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Actinomyces	0,0001865	0,00015	1,82E-05	0,0001	4E-05
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Actinotignum	0	0	0	0	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Arcanobacterium	0	0	0	0	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Mobiluncus	3,243E-05	0	0	9,6E-05	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Varibaculum	0	0	0	2,5E-05	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__uncultured	0	4,7E-06	0	0	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Aeriscardovia	0	0	0	0	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Alloscardovia	1,621E-05	4,7E-06	0	0	0
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Bifidobacterium	0,0268425	0,04133	0,000249	0,10931	0,0093
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Gardnerella	0	9,5E-06	0	5E-05	5E-06
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Pseudoscardovia	0	4,7E-06	0	0	0

D0 = kingdom
D1 = phylum
D2 = class
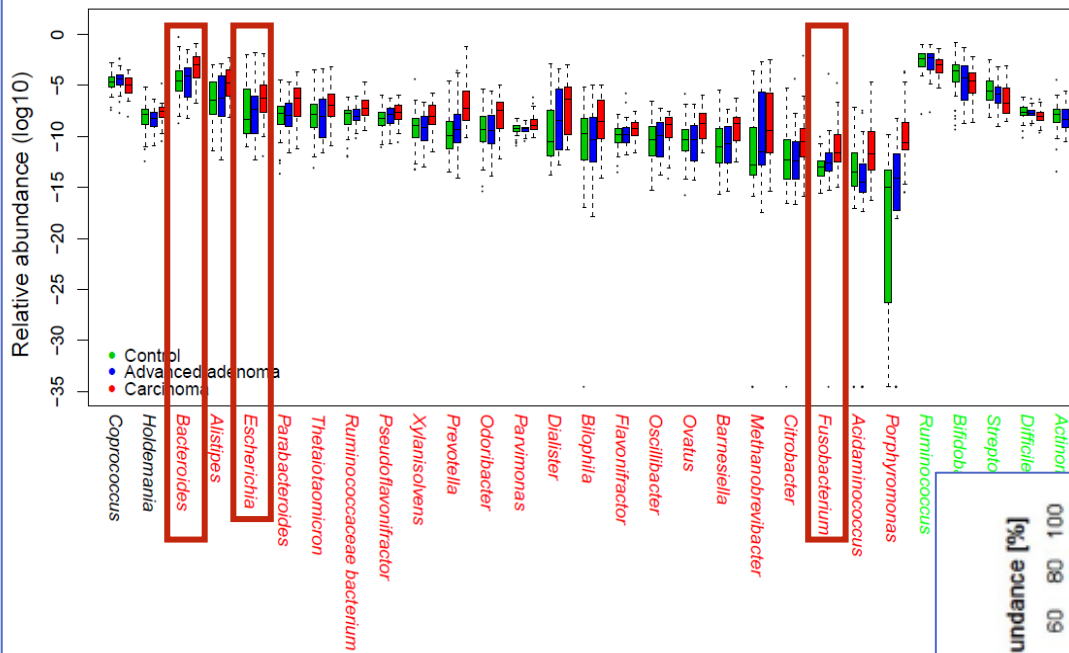**D3** = order
D4 = family
D5 = genus

OTU Table

Bioinformatic and Statistical analysis
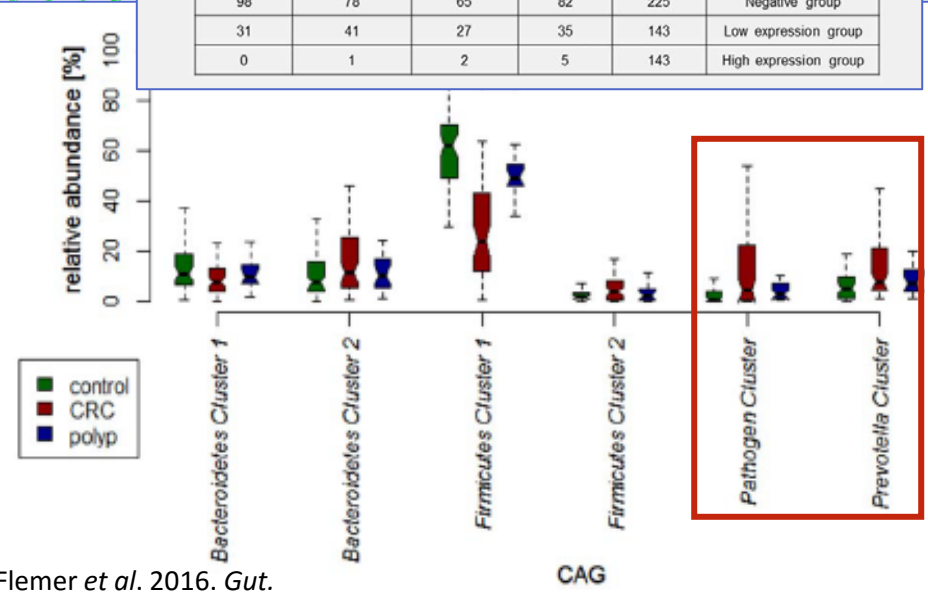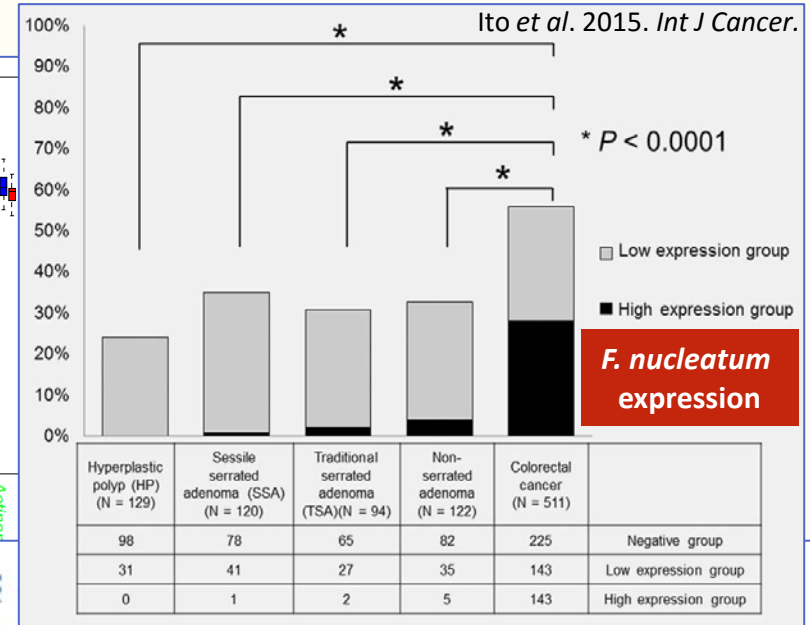
Taxonomy charts
Relative abundance of taxonomic groups

Percentage distribution of taxa — Samples

Legend:
p_Synergistetes
Other_Bacteria
p_Verrucomicrobia
p_Tenericutes
p_Cyanobacteria
p_Actinobacteria
p_Proteobacteria
p_Bacteroidetes
p_Firmicutes

Gut microbiome and colorectal adenoma-carcinoma sequence

Feng *et al*. 2015. *Nature Comm.*

Ito *et al*. 2015. *Int J Cancer.*

F. nucleatum expression

* *P* < 0.0001

	Hyperplastic polyp (HP) (N = 129)	Sessile serrated adenoma (SSA) (N = 120)	Traditional serrated adenoma (TSA)(N = 94)	Non-serrated adenoma (N = 122)	Colorectal cancer (N = 511)	
	98	78	65	82	225	Negative group
	31	41	27	35	143	Low expression group
	0	1	2	5	143	High expression group

Flemer *et al*. 2016. *Gut.*

Study of gut microbiome dysbiosis in the progression of neoplasia in sporadic colorectal cancer (CRC)

- **Istituto Scientifico Romagnolo per la Cura e lo Studio dei Tumori** (IRST), Meldola
- **Department of Biology and Biotechnology «L. Spallanzani»**, University of Pavia, Pavia
- **Centre for Integrative Biology**, CIBIO, University of Trento, Trento.

96 stool samples

Stage of neoplastic progression	N° patients
Healthy (negative colonscopy)	19
Hyperplastic polyps (HPs)	14
Low risk adenomas (LRAs)	20
High risk adenomas (HRAs)	21
Carcinomas (ADKs)	13
ADKs treated with chemotherapy/radiotherapy	9

Bacterial DNA extraction

↓

V4 16S rRNA libraries

↓

Illumina sequencing

↓

Bioinformatic analysis

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In progress…

- We are waiting for **sequencing results and bioinformatic analysis of all samples**.
- Microbiome composition will be characterized for entire **colorectal cancerogenesis progression**.
- We will put in **correlation patients' microbiome with their clinical data**.

FINAL GOALS

- To identify microbial population that are specific to **early time points prior to disease development**.
- Dysbiotic patients can be treated to **restore eubiosis** **(personalized medicine)**.

Future perspectives

- **Metabolomics** and **mycobiome** study.
- Correlation with **diet** and nutrition.
- Microbiome dysbiosis in **colorectal cancer hereditary syndromes**.

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