

# Canvis induïts a la microbiota després del tractament amb biològics: Beneficiosos o perjudicials ?

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# Microbioma humà



## Our Microbiome

In our bodies human cells are outnumbered ten to one by bacteria. Some eight million genes function in this invisible universe—more than 300 times the number in our own cells. Though some of our microbial tenants pose threats, we literally can't live without most of them. They help digest our food, guide our immune system, and ward off deadly germs.

### THE BODY'S NEIGHBORHOODS

Different regions of our body have unique populations of bacteria, some more diverse than others.

Four species of bacteria

### THROAT

4,154 species

Major player:  
*Neisseria lactamica*  
Babies have more of this microbe than adults, perhaps because it may help build immunity against meningitis.

### BEHIND THE EARS

2,359 species

Major player:  
*Propionibacterium acnes*  
Although associated with acne, this bacterium also inhibits the growth of fungi and yeast on the skin.

### NOSTRILS

2,264 species

Major player:  
*Staphylococcus epidermidis*  
This species keeps the nostrils' teeming bacterial colonies in equilibrium and suppresses dangerous strains of staph.

### TONGUE

7,947 species

Major player:  
*Streptococcus salivarius*  
This bacterium is an ally, helping prevent tooth decay, gum disease, and throat infections.

### INNER ELBOWS

2,012 species

Major player:  
*Corynebacterium similians*  
Generally beneficial, this species has antimicrobial properties that inhibit or kill more harmful pathogens.

### VAGINAL OPENING

2,062 species

Major player:  
*Lactobacillus acidophilus*  
Lactobacillus produces lactic acid, which maintains a low pH and inhibits the growth of harmful bacteria.

### BABY'S FIRST BUGS

The microbes that colonize an infant "teach" the immune system as it develops in the first three years of life and influence the baby's risk of allergies, eczema, and more.

Microbes from the mother's vagina make it easier for a newborn to live on the outside. Bacteria in the Lactobacillales order (bright blue) help the baby digest milk.



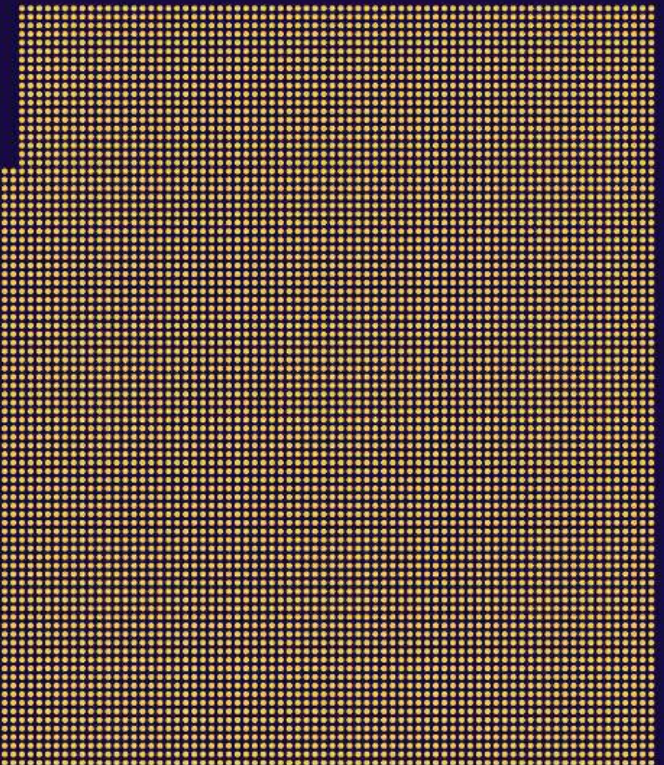
MICROBIAL DIVERSITY ON NEWBORN'S SKIN  
Each color represents an order of bacteria.

C-section babies have fewer lactobacillus bacteria and more potentially harmful microbes picked up from adult skin, including staphylococcus and Acinetobacter.

### LARGE INTESTINE\*

33,627 species

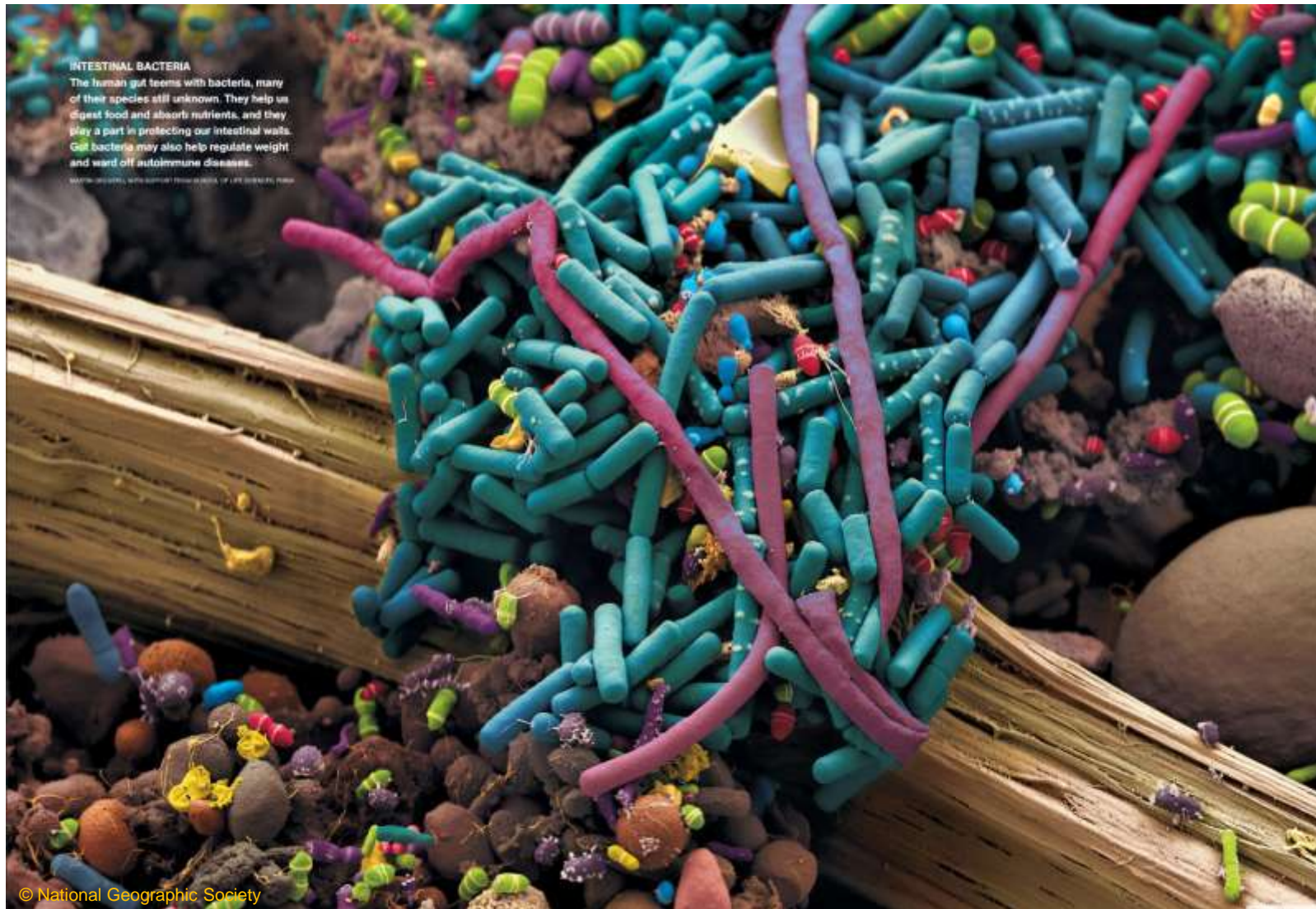
Major player:  
*Bacteroides thetaiotaomicron*  
This microbe digests starches from plants, allowing infants to shift from mother's milk to table food.



\*Data taken from stool sample

GRAPHIC: LAWSON PARKER, NGM STAFF; SOURCES: SUSAN M. HUSE, "A CORE HUMAN MICROBIOME AS VIEWED THROUGH 1165 RRNA SEQUENCE CLUSTERS" (SPECIES COUNT); MARIA D. DOMINGUEZ-BELLO, "DELIVERY MODE SHAPES THE ACQUISITION AND STRUCTURE OF THE INITIAL MICROBIOTA ACROSS MULTIPLE BODY HABITATS IN NEWBORNS" (NEWBORN DATA)

# Bactèria & cos humà: Bactèries intestinals

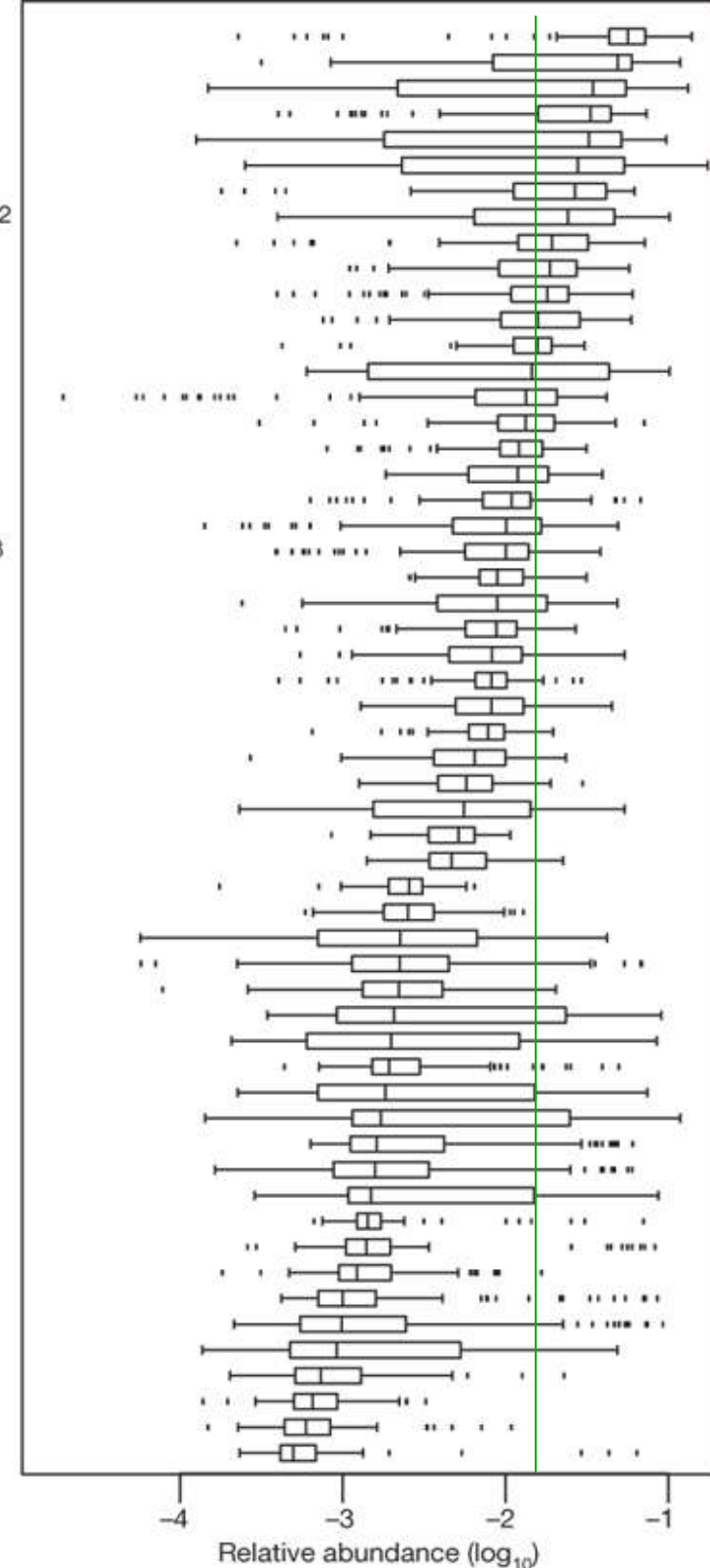


# Nucli de la microbiota

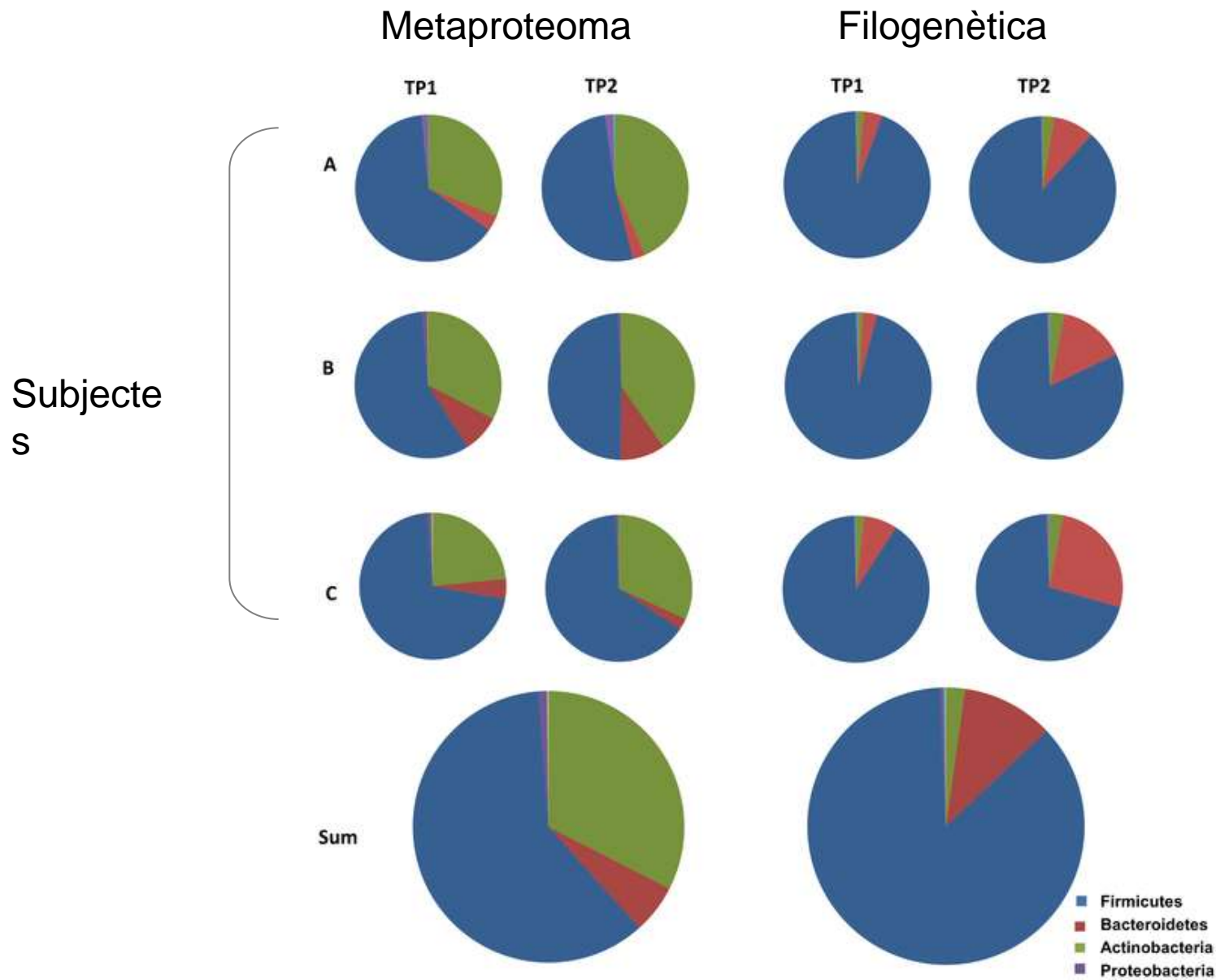
Abundància relativa dels genomes microbials més freqüents entre individus de la cohort.

JJ Qin *et al. Nature* **464**, 59-65 (2010)  
doi:10.1038/nature08821

*Bacteroides uniformis*  
*Alistipes putredinis*  
*Parabacteroides merdae*  
*Dorea longicatena*  
*Ruminococcus bromii* L2-63  
*Bacteroides caccae*  
*Clostridium* sp. SS2-1  
*Bacteroides thetaiotaomicron* VPI-5482  
*Eubacterium hallii*  
*Ruminococcus torques* L2-14  
 Unknown sp. SS3 4  
*Ruminococcus* sp. SR1 5  
 → *Faecalibacterium prausnitzii* SL3 3  
*Ruminococcus lactaris*  
*Collinsella aerofaciens*  
*Dorea formicigenerans*  
*Bacteroides vulgatus* ATCC 8482  
*Roseburia intestinalis* M50 1  
*Bacteroides* sp. 2\_1\_7  
*Eubacterium siraeum* 70 3  
*Parabacteroides distasonis* ATCC 8503  
*Bacteroides* sp. 9\_1\_42FAA  
*Bacteroides ovatus*  
*Bacteroides* sp. 4\_3\_47FAA  
*Bacteroides* sp. 2\_2\_4  
*Eubacterium rectale* M104 1  
*Bacteroides xylanisolvens* XB1A  
*Coprococcus comes* SL7 1  
*Bacteroides* sp. D1  
*Bacteroides* sp. D4  
*Eubacterium ventriosum*  
*Bacteroides dorei*  
*Ruminococcus obeum* A2-162  
*Subdoligranulum variabile*  
*Bacteroides capillosus*  
*Streptococcus thermophilus* LMD-9  
*Clostridium leptum*  
*Holdemania filiformis*  
*Bacteroides stercoris*  
*Coprococcus eutactus*  
*Clostridium* sp. M62 1  
*Bacteroides eggerthii*  
*Butyrivibrio crossotus*  
*Bacteroides finegoldii*  
*Parabacteroides johnsonii*  
*Clostridium* sp. L2-50  
*Clostridium nexile*  
*Bacteroides pectinophilus*  
*Anaerotruncus colihominis*  
*Ruminococcus gnavus*  
*Bacteroides intestinalis*  
*Bacteroides fragilis* 3\_1\_12  
*Clostridium asparagiforme*  
*Enterococcus faecalis* TX0104  
*Clostridium scindens*  
*Blautia hansenii*



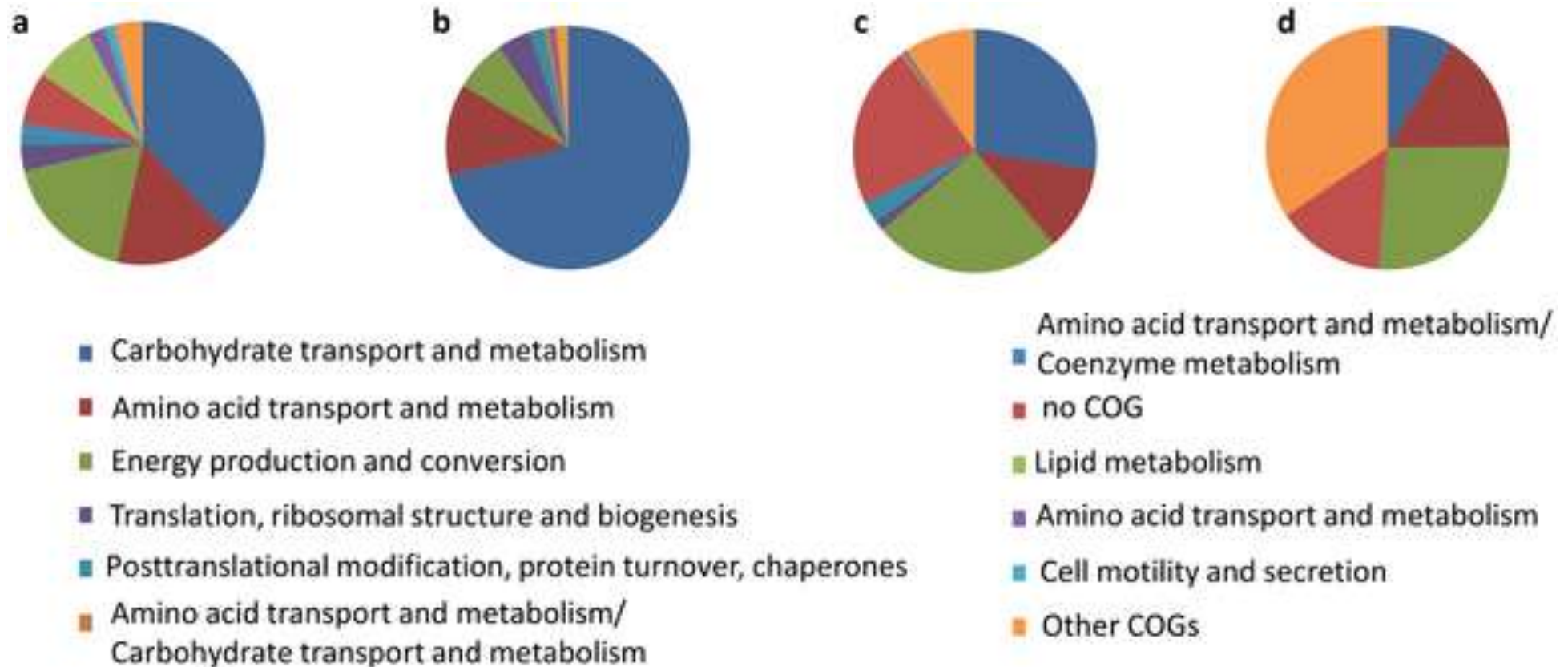
# Microbiota principal i funcions principals



Kolmeder CA, de Been M, Nikkilä J, Ritamo I, et al. (2012) Comparative Metaproteomics and Diversity Analysis of Human Intestinal Microbiota Testifies for Its Temporal Stability and Expression of Core Functions. PLoS ONE 7(1): e29913. doi:10.1371/journal.pone.0029913  
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0029913>

# Microbiota principal: Funcionalsisme

a) Firmicutes, b) Actinobacteria, c) Bacteroidetes and d) Proteobacteria.



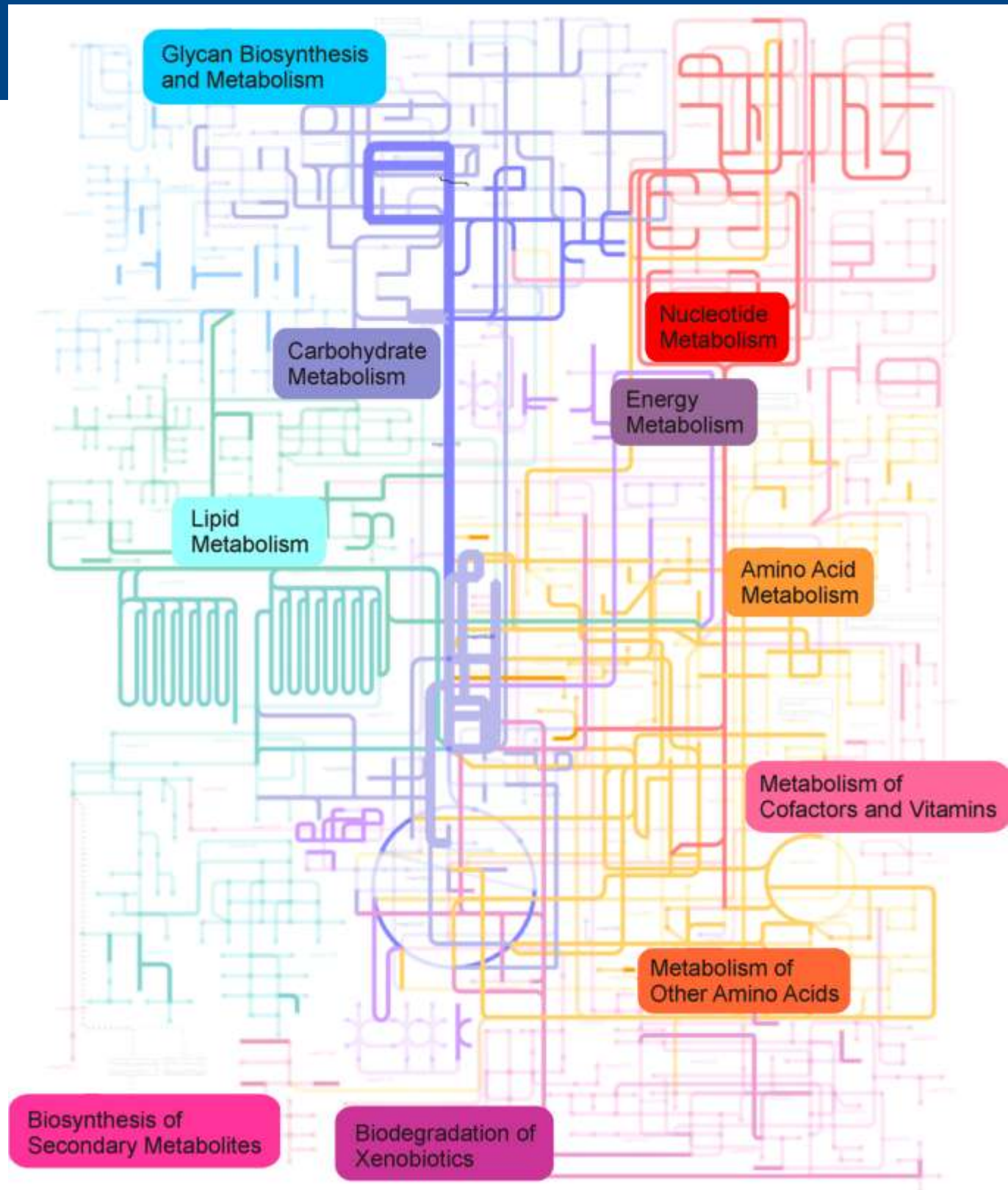
Kolmeder CA, de Been M, Nikkilä J, Ritamo I, et al. (2012) Comparative Metaproteomics and Diversity Analysis of Human Intestinal Microbiota Testifies for Its Temporal Stability and Expression of Core Functions. PLoS ONE 7(1): e29913.

doi:10.1371/journal.pone.0029913

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0029913>

Faecal metaproteome is subject-specific and **stable** during a one-year period.

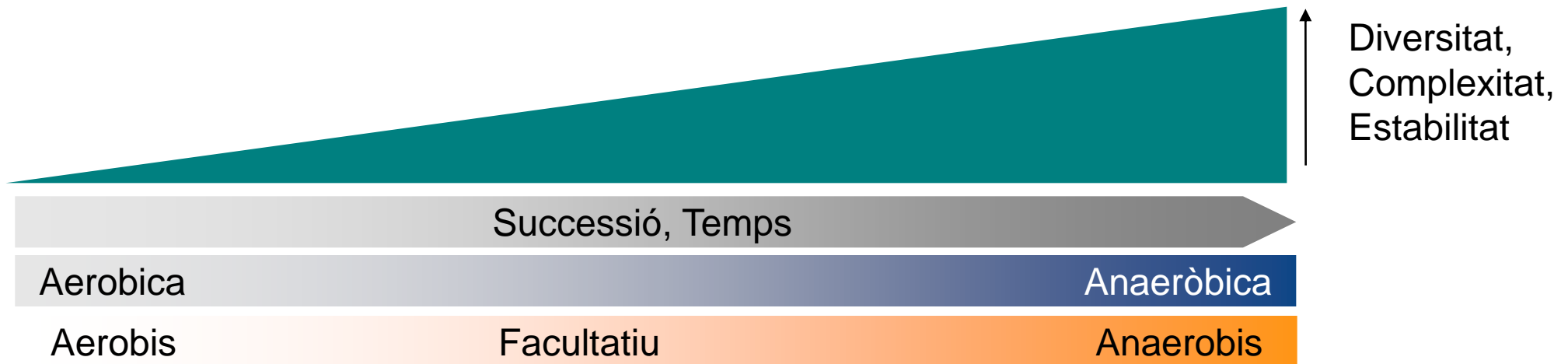
A **stable common core** of approximately 1,000 proteins could be recognized in each of the subjects, indicating a common functional core that is mainly involved in carbohydrate transport and degradation



# Un pèl d'Ecologia

- Colonitzados
- Bactèries generalistes
- Alts índexs de creixement

- Especialitzats
- Índexs de creixement baixos



- Ric en nodrients
- Components orgànics d'alt pes molecular

- Fermentació
- Components orgànics de baix pes molecular



# Un pèl d'Ecologia

Aerobis

Facultatius

Anaerobis

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- Bactèries generalistes
- Alts índexs de creixement

**PROTEOBACTERIA**

- Especialitzats
- Índexs de creixement baixos

UC, CD?

Disbiosi

**Perturbació**

**Recuperació**

UC, CD?

Curació mucosa

Successió secundària

# Un pèl d'Ecologia

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Disbiosi

**Perturbació**

**On s'ubica la immunomodulació en aquest esquema?**

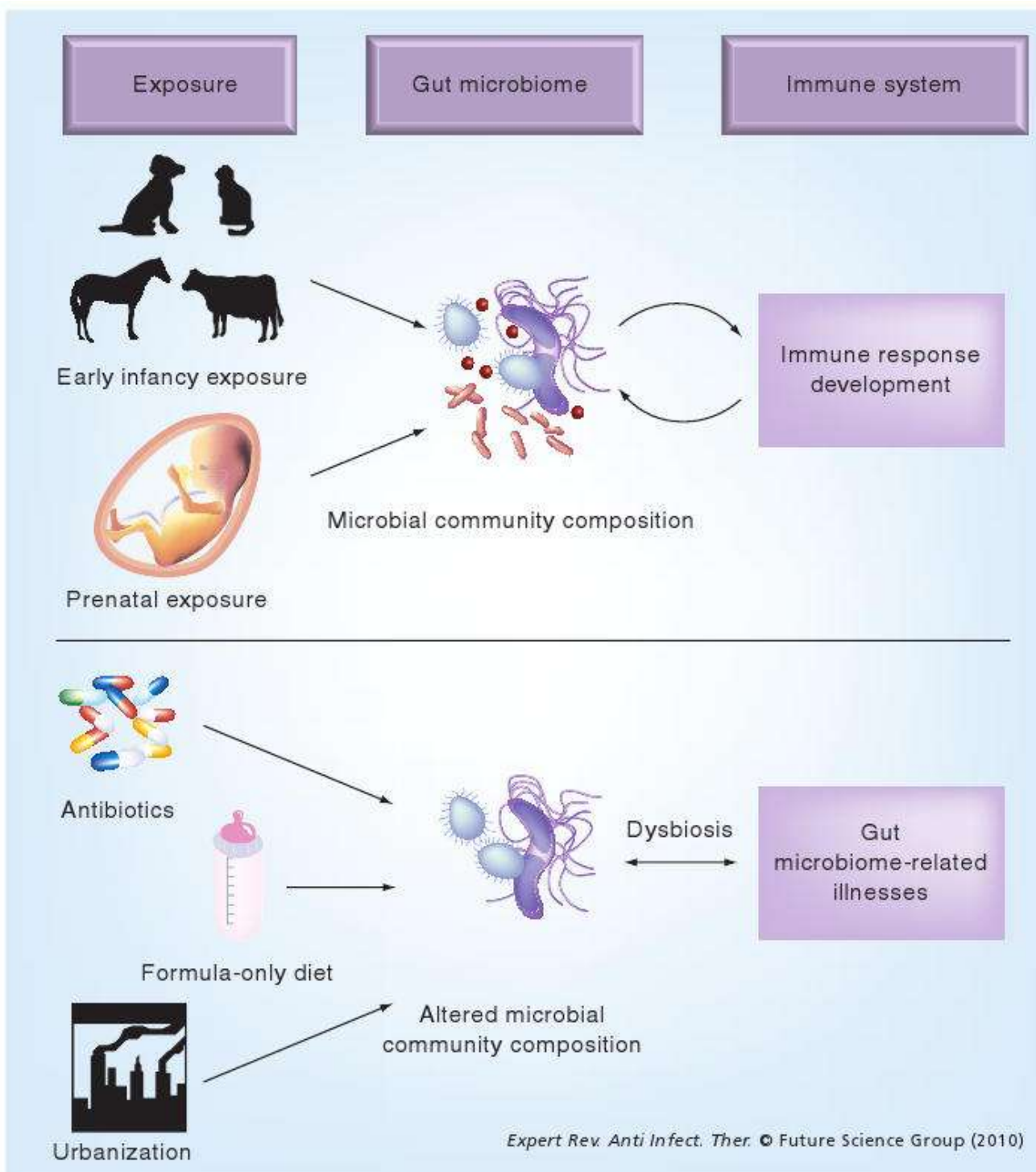
**Recuperació**

UC, CD?

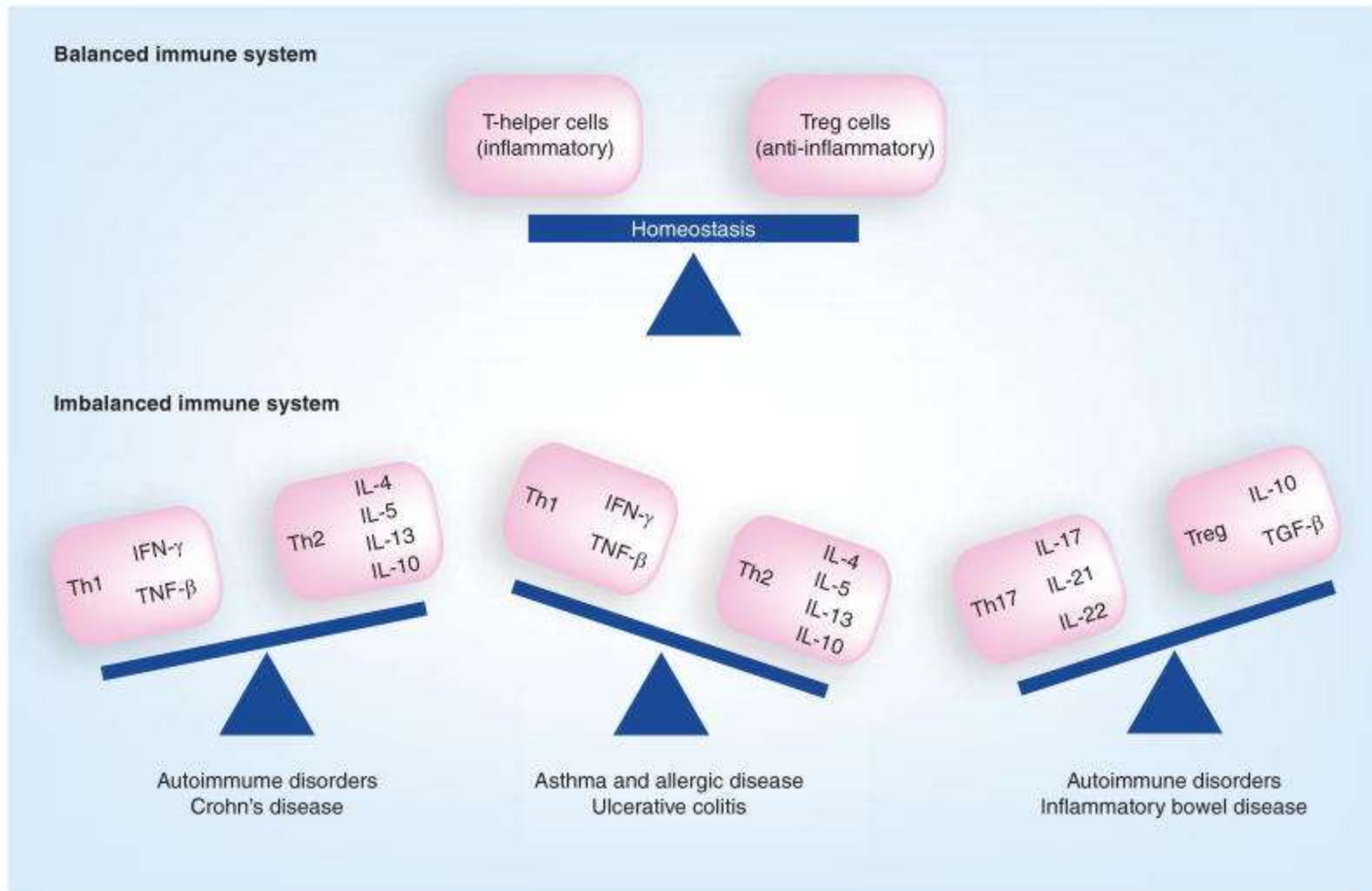
Curació mucosa"

Succesió secundària

# Bactèries i immunitat



# Alteració balanç immunològic a MII



# Alteracions en la composició bacteriana en MII: MC

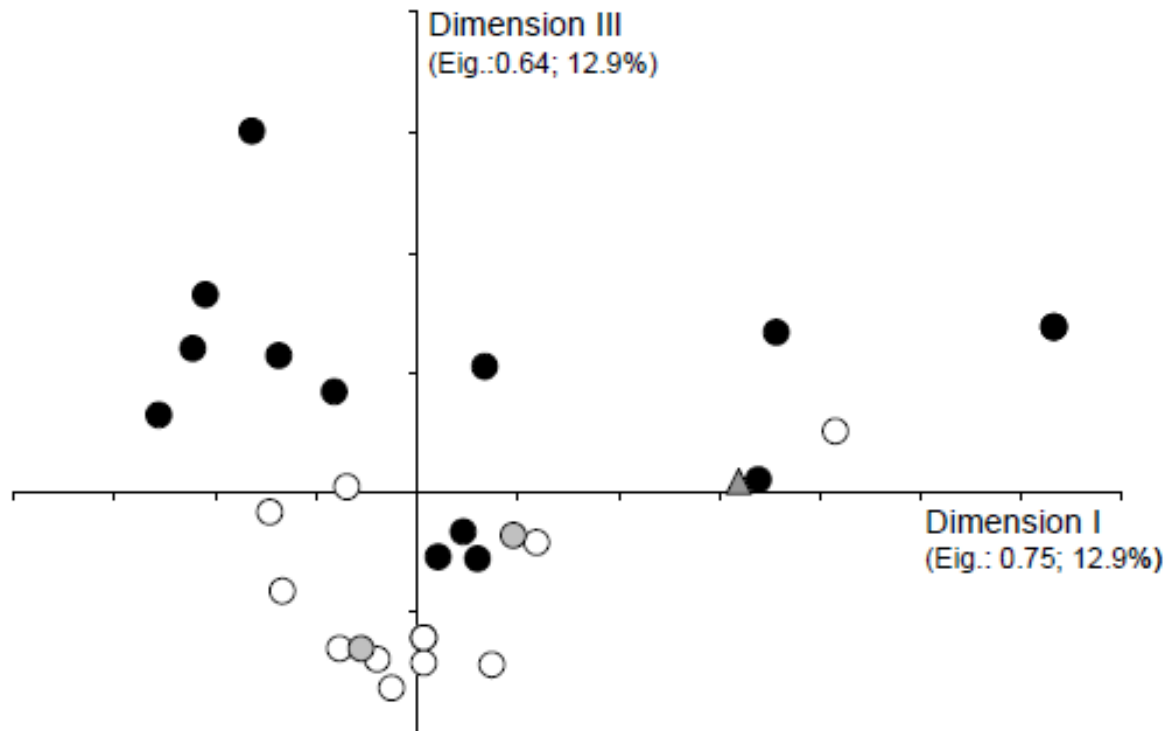
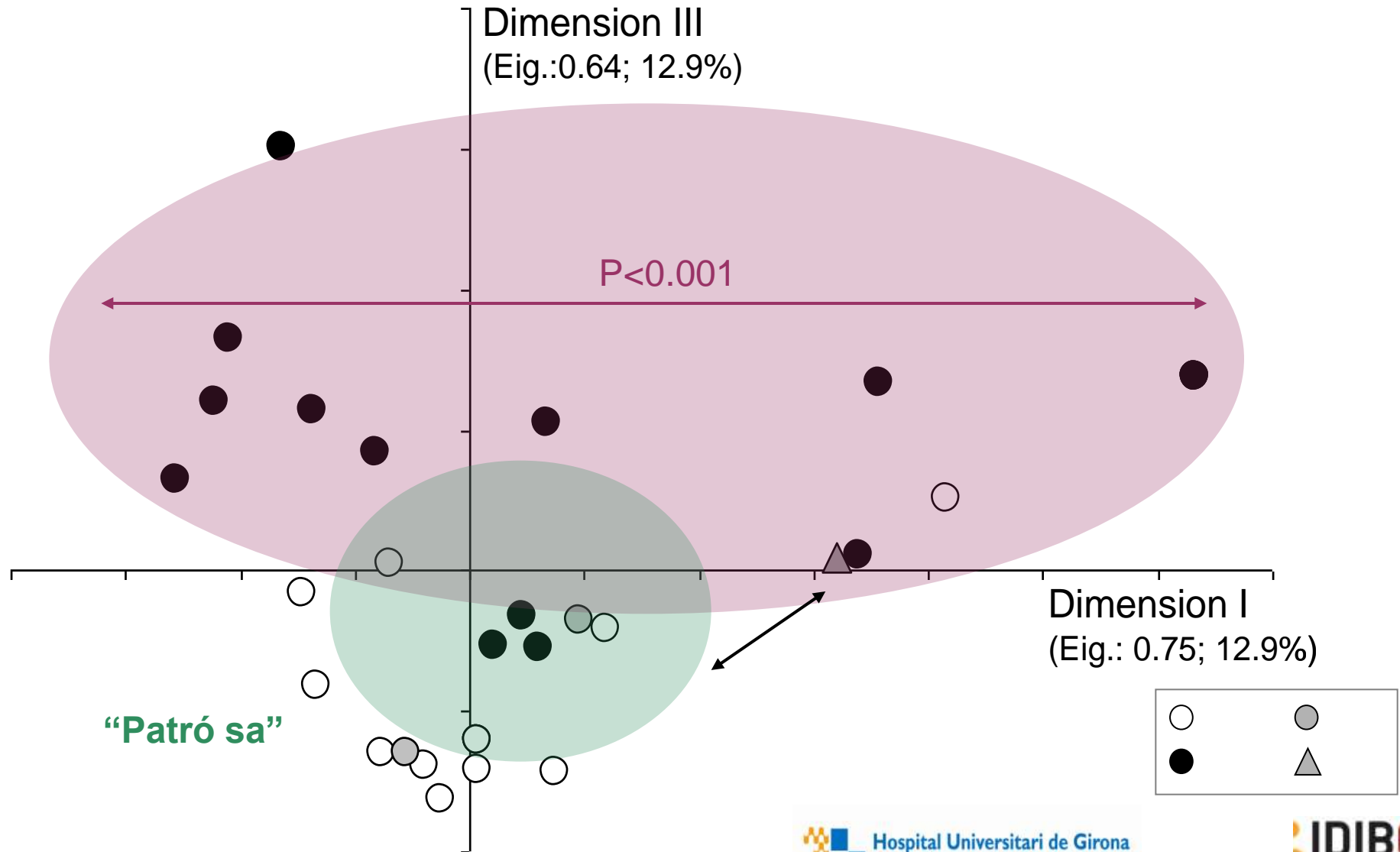


Figure 14. Correspondence analysis using DGGE profiles. ○: non-IBD controls; ●: Crohn's disease (CD) patients; ●: Ulcerative colitis (UC); ▲: ischemic colitis (IC). Dimension 3 separates specimens according to their diagnostic ( $P < 0.001$ ) and dimension 1 displays the higher DGGE profile dispersion within CD patients.

M Martinez-Medina, et al, (2006) Inflammatory bowel diseases 12 (12), 1136-1145

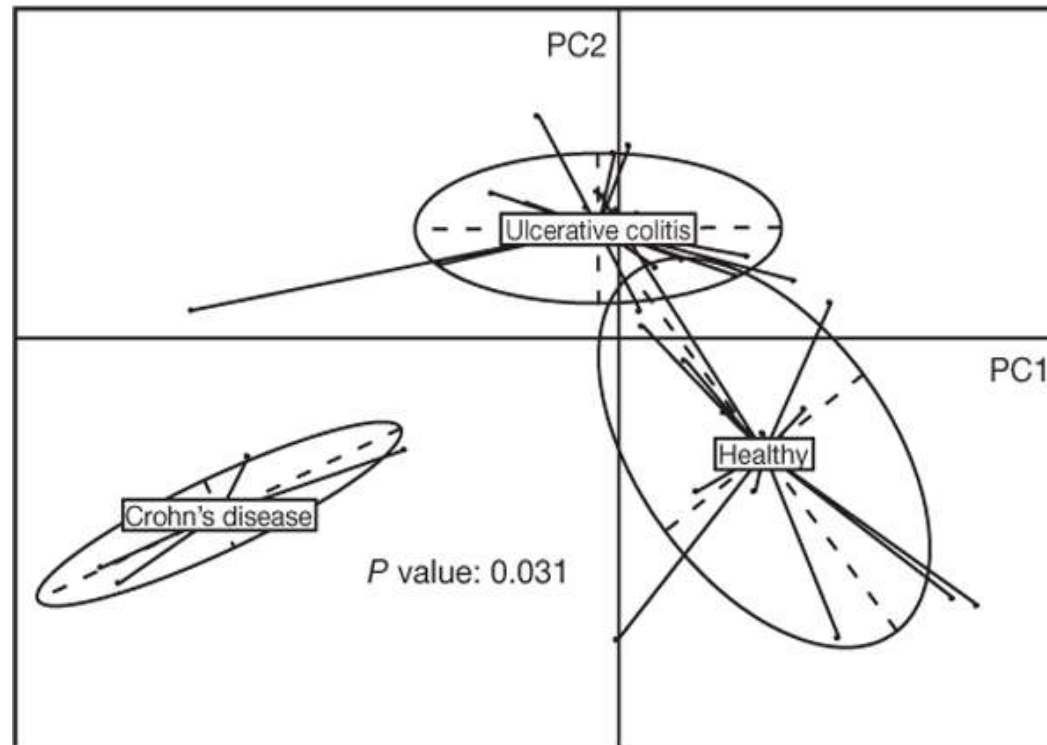
## Anàlisi de correspondències dels filotips recuperats

### Major variabilitat interpacient dins de la MC



# Alteracions en la composició bacteriana en MII: MC i CU

Bacterial species abundance differentiates IBD patients and healthy individuals.



JJ Qin *et al.* *Nature* **464**, 59-65 (2010) doi:10.1038/nature08821

nature

# Efecte dels biològics: Infiximab. Tècnica FISH.

group	Healthy controls	IBD remission	CD-active (n=4)		CD1		CD2		CD3		CD4	
	(n=7)	(n=11)	before	after	before	after	before	after	before	after	before	after
<i>Bacteroides/Prevotella</i> group	6.7 (3.4-26.1)	6.9 <sup>†</sup> (0.4-21.7)	17.5 <sup>**</sup> (12.3-53.2)	29.9 <sup>#</sup> (12.7-34.6)	16.1	28.0	18.9	31.9	53.2	12.7	12.3	34.6
<i>Eubacterium rectale/C. coccoides</i> group	14.6 (6.0-45.8)	22.6 (1.7-50.2)	24.2 (6.7-45.5)	18.9 (15.6-35.2)	6.7	15.6	22.0	35.2	26.4	18.2	45.5	19.5
<i>Bifidobacterium</i>	4.2 (0.7-6.3)	1.8 (0.4-11.1)	3.7 (1.4-7.9)	1.7 (0.7-12.4)	1.4	1.3	2.3	2.2	5.0	12.4	7.9	0.7
low G+C Gram-positive bacteria	8.8 (0.3-12.6)	5.3 (0.4-12.2)	2.6 (0.1-23.9)	3.8 (0.8-11.9)	4.9	6.1	0.4	1.5	23.9	11.9	0.1	0.8
<i>Atopobium</i> group	2.9 (0.1-7.5)	3.5 (1.4-7.2)	0.9 (0.2-4.3)	0.4 (<0.1-4.9)	1.1	0.4	4.3	4.9	0.2	0.2	0.8	<0.1
<i>C. ramosum</i>	0.6 (0.2-1.8)	0.6 (0.0-4.2)	0.9 (<0.5-2.9)	0.8 (0.2-1.5)	<0.5	0.2	0.5	0.7	2.9	1.5	1.0	1.0
<i>C. spiroforme/C. cocleatum</i>	0.1 (0.0-0.2)	0.2 (0.1-0.4)	0.1 (<0.1-0.3)	0.1 (<0.1-0.3)	<0.5	0.1	<0.1	0.1	0.3	0.3	0.1	<0.1
<i>C. histolyticum/C. lituseburensis</i> group	0.2 (0.1-0.2)	0.2 (0.0-0.1)	0.2 (0.2-0.6)	0.1 (<0.1-0.6)	<0.5	<0.1	0.2	0.1	0.2	0.1	0.6	0.6
<i>Lactobacillus/Enterococcus</i>	0.0 (0.0-0.1)	0.1 (0.0-0.1)	0.02 (<0.1-0.5)	0.05 (<0.1-0.1)	<0.5	<0.1	<0.1	0.1	<0.5	<0.1	<0.5	<0.1
<i>Streptococcus/Lactococcus</i>	1.1 (0.3-1.8)	0.3 (0.1-0.4)	0.5 (0.2-3.1)	1.1 (0.2-2.5)	0.4	0.3	0.6	1.7	0.2	0.5	3.1	2.5
unidentified bacteria	57.6 (23.9-79.0)	50.8 (30.7-87.6)	39.8 (0-69.4)	41.2 (21.6-48.1)	69.4	48.1	50.9	21.6	-	42.3	28.8	40.0

# p≤0.05 healthy controls versus CD-after

† p≤0.05 IBD-remission versus CD-after

\*\* p≤0.05 IBD-remission versus CD-before

before/after=before/after infliximab treatment



# Efecte dels biològics: Adalimumab a la microbiota (I)

Biopsies:  
rectoscopy

Ultra-sound  
washes:  
Discard transient  
and loosely  
attached bacteria

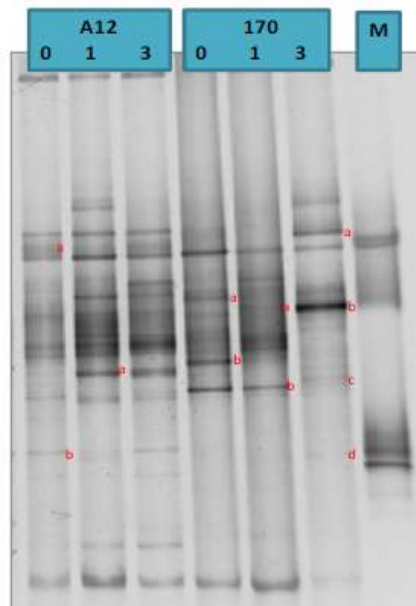
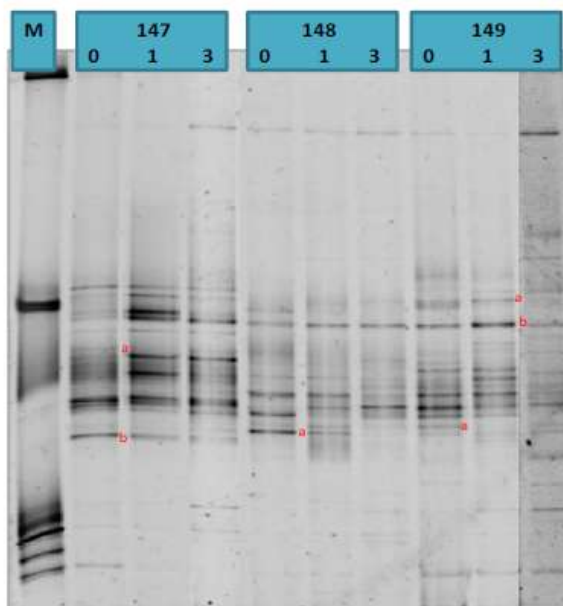
DNA extraction  
and purification

PCR / DGGE  
*Eubacteria*  
GC-357f-907r<sup>2</sup>

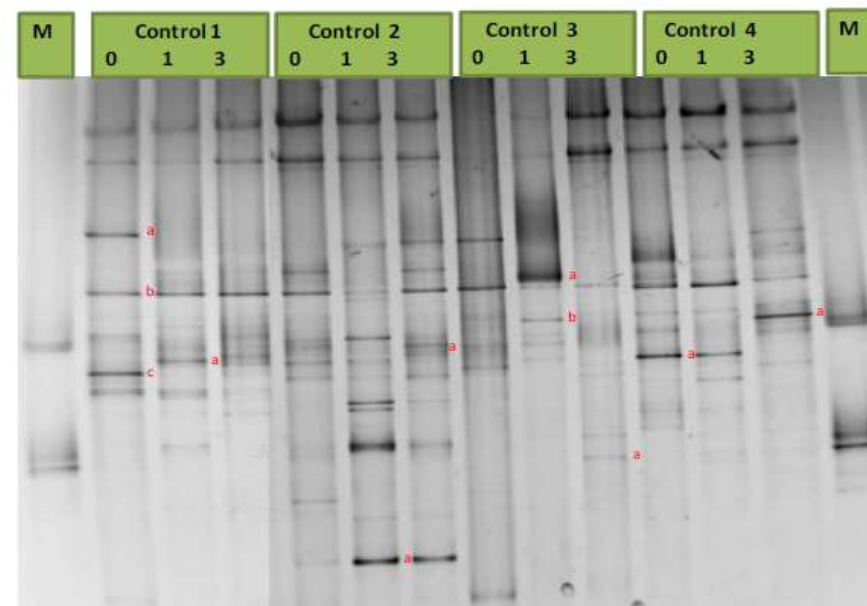
DNA  
reamplification &  
sequencing

Sequence analysis  
GenBank / RDP

CD patients



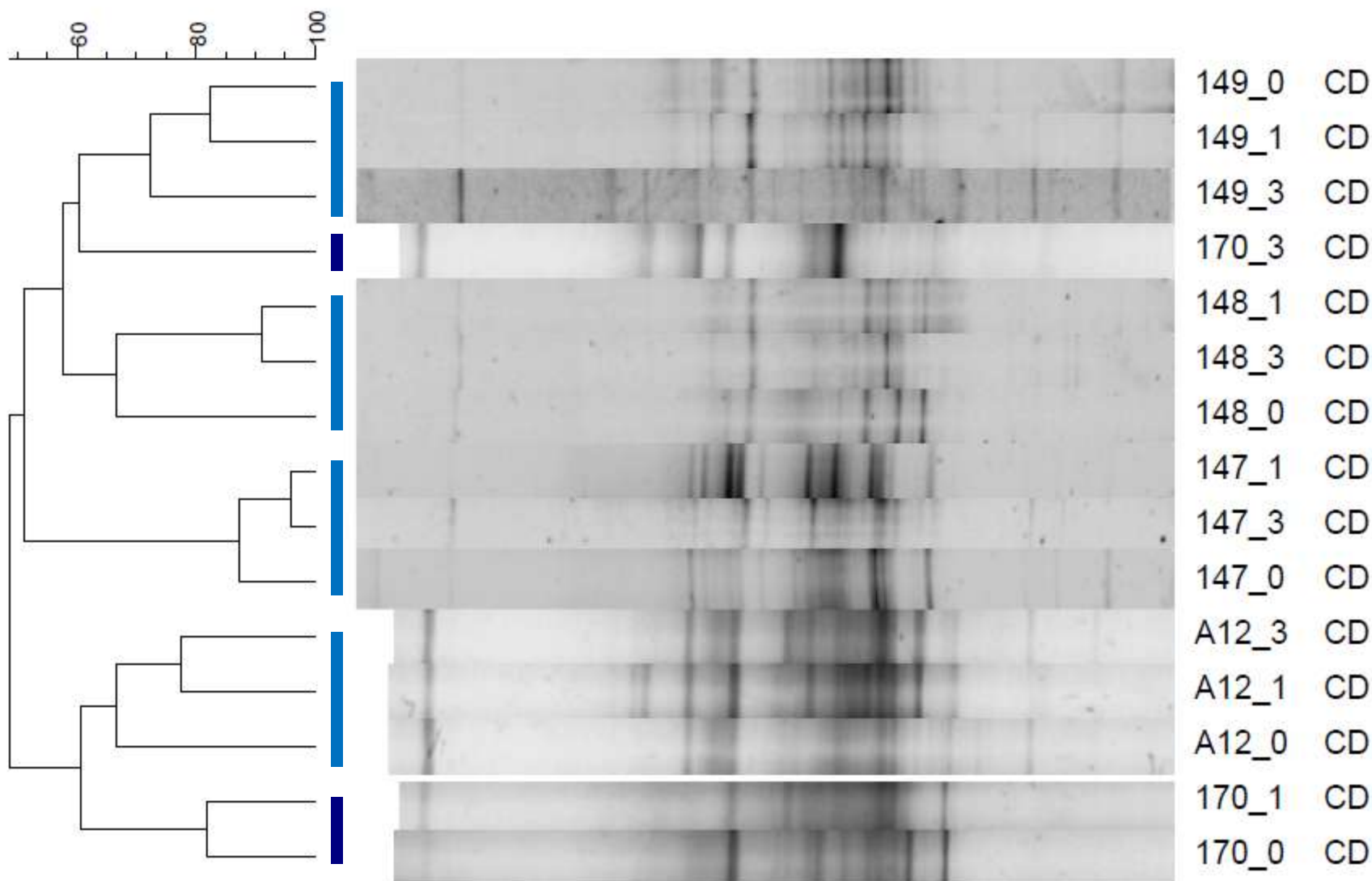
Controls



# Efecte dels biològics: Adalimumab a la microbiota(II)

DGGE band	Size	Accession	Description	Max ident	Source	S_ab	Strain RDP-II	Accession2
148(0)a	437	<a href="#">DQ350809.1</a>	Uncultured Clostridia bacterium clone Clept59 16S	90%	human feces	<0.6	no significant match	
A12(0)b	510	<a href="#">AY333095.1</a>	Uncultured bacterium clone 3-3	91%	enviromental sample	<0,5		
147(0)b	132	<a href="#">EF405503.1</a>	Uncultured bacterium clone SJTU_G_06_62	96%	human fecal sample	0.762	<i>Phascolarctobacterium faecium</i>	<a href="#">X72867</a>
A12(1)a	526	<a href="#">DQ802194.1</a>	Uncultured bacterium clone RL241_aaj04c09	96%	human feces	0,849	butyrate-producing bacterium SR1/1; AY305321	<a href="#">S000406447</a>
C2(1)a	460	<a href="#">AY738685.1</a>	Uncultured <i>Prevotella</i> sp. clone 136-b 40	96%	Homo sapiens vagina	0.774	<i>Prevotella buccalis</i>	<a href="#">L16476</a>
C2(3)b	553	<a href="#">DQ794406.1</a>	Uncultured bacterium clone RL197_aah87d01	96%	human feces	0.780	<i>Roseburia faecalis</i>	<a href="#">AY804150</a>
149(1)b	476	<a href="#">DQ798468.1</a>	Uncultured bacterium clone RL181_aan97b02	97%	human feces	0.838	<i>Faecalibacterium prausnitzii</i>	<a href="#">AJ270469</a>
149(1)a	543	<a href="#">DQ825272.1</a>	Uncultured bacterium clone RL185_aan87f02	98%	human feces	0.900	<i>Faecalibacterium prausnitzii</i>	<a href="#">AJ270469</a>
A12(0)a	520	<a href="#">EU768790.1</a>	Uncultured bacterium clone C5_772	98%	fecal sample	0,955	<i>Bacteroides dorei</i> (T); JCM 13471; 175; AB242142	<a href="#">S000650604</a>
170(0)a	509	<a href="#">EU467277.1</a>	Uncultured bacterium clone SPIM_f03	98%	black-handed spider monkey feces	0,65	<i>Prevotellaceae</i> bacterium P4P_62 P1; AY207061	<a href="#">S000401697</a>
170(1)b	515	<a href="#">AM075720.1</a>	Uncultured bacterium isolate CD0715	98%	intestinal mucosa from Crohn's disease patient	0,847	<i>Catenibacterium mitsuokai</i> ; JCM 10606; AB030221	<a href="#">S000018370</a>
170(3)a	520	<a href="#">EF399597.1</a>	Uncultured bacterium clone SJTU_F_12_45	98%	human fecal sample	0,922	<i>Clostridium innocuum</i> (T); M23732	<a href="#">S000436096</a>
170(3)b	519	<a href="#">EU767107.1</a>	Uncultured bacterium clone C1_136	98%	fecal sample	0.792	butyrate-producing bacterium A2-231; AJ270484	<a href="#">S000089036</a>
C3(1)a	534	<a href="#">EU768790.1</a>	Uncultured bacterium clone C5_772	98%	fecal sample	0.938	<i>Bacteroides vulgatus</i>	<a href="#">EU136687</a>
147(0)a	563	<a href="#">AB382610.1</a>	Uncultured bacterium clone: KC-C-078	99%	piglet caecum	0.99	<i>Ruminococcus bromii</i>	<a href="#">X85099.1</a>
149(0)a	633	<a href="#">DQ905754.2</a>	Uncultured bacterium clone 29A-e9	99%	Homo sapiens fecal sample	0.793	<i>Ruminococcus luti</i>	<a href="#">AJ133124</a>
170(0)b	517	<a href="#">FJ375931.1</a>	Uncultured bacterium clone PBM_b23	99%	polar bear feces	0,94	<i>Ruminococcus gnavus</i> ; ATCC 29149; L76597	<a href="#">S000436013</a>
170(1)a	530	<a href="#">EU778318.1</a>	Uncultured bacterium clone RL388_aao93f08	99%	mammal feces	0,901	<i>Prevotella</i> sp. DJF_B112; EU728712	<a href="#">S001046004</a>
170(3)d	518	<a href="#">AF227828.1</a>	<i>Corynebacterium</i> sp. 61722	99%		0,937	<i>Corynebacterium</i> sp. 61722; AF227828	<a href="#">S000389537</a>
C1(0)a	561	<a href="#">M23731.1</a>	<i>C. ramosum</i>	99%		0.975	<i>Clostridium ramosum</i>	<a href="#">AY699288</a>
C1(0)b	563	<a href="#">EU768619.1</a>	Uncultured bacterium clone C5_205	99%	fecal sample	0.980	<i>Faecalibacterium prausnitzii</i>	<a href="#">AJ270470</a>
C1(0)c	561	<a href="#">EU767911.1</a>	Uncultured bacterium clone C3_365	99%	fecal sample	0.978	<i>Ruminococcus gnavus</i>	<a href="#">X94967</a>
C1(1)a	564	<a href="#">EF405388.1</a>	Uncultured bacterium clone SJTU_G_01_13	99%	fecal sample	0.800	<i>Clostridium populeti</i>	<a href="#">X71853</a>
C4(0)a	562	<a href="#">EU530458.1</a>	Uncultured Clostridiaceae bacterium clone M7-25	99%	adenoma colorectal cancer	0.791	<i>Clostridium leptum</i>	<a href="#">AJ305238</a>
C4(3)b	565	<a href="#">AF357574.1</a>	Bacterium mpn-isolate group 26	99%	feces of post-weaning piglets	0.982	bacterium mpn-isolate group 26	<a href="#">AF357574</a>
170(3)c	535	<a href="#">EU767133.1</a>	Uncultured bacterium clone C1_253	100%	fecal sample	0,92	<i>Clostridium nexile</i> (T); DSM 1787; X73443	<a href="#">S000260729</a>
C3(1)b	559	<a href="#">EU766369.1</a>	Uncultured bacterium clone B5_029	100%	fecal sample	0.998	<i>Clostridiaceae</i> bacterium	<a href="#">EU183300</a>
C3(3)a	559	<a href="#">EU821777.1</a>	<i>Micrococcus</i> sp. SBS-8	100%		1.000	<i>Micrococcus luteus</i>	<a href="#">AJ717367</a>

# Efecte dels biològics: Adalimumab a la microbiota (III)



- Espècies conegudes que són més prevalents en MC com ***Ruminococcus*** spp i ***Clostridium*** spp. Desapereixen o es redueixen considerablement com es revela per les 17 seqüències analitzades dels pacients d'aquest estudi. Altres com ***Bacteroides*** spp apareixien així com bandes de l'indicador de microbiota sana ***Faecalibacterium prausnitzii*** que incrementen la seva intensitat.
- Aquests canvis en la microbiota van apuntar a una **recuperació parical de la microbiota normal** i les condicions fisiològiques del còlon.
- El tractament amb Adalimumab semblen introduir **canvis a curt plaç** que podrien ésser **beneficiosos**. Aquests canvis poden ésser d'interès per monitorar l'efectivitat del tractament en períodes més llargs.

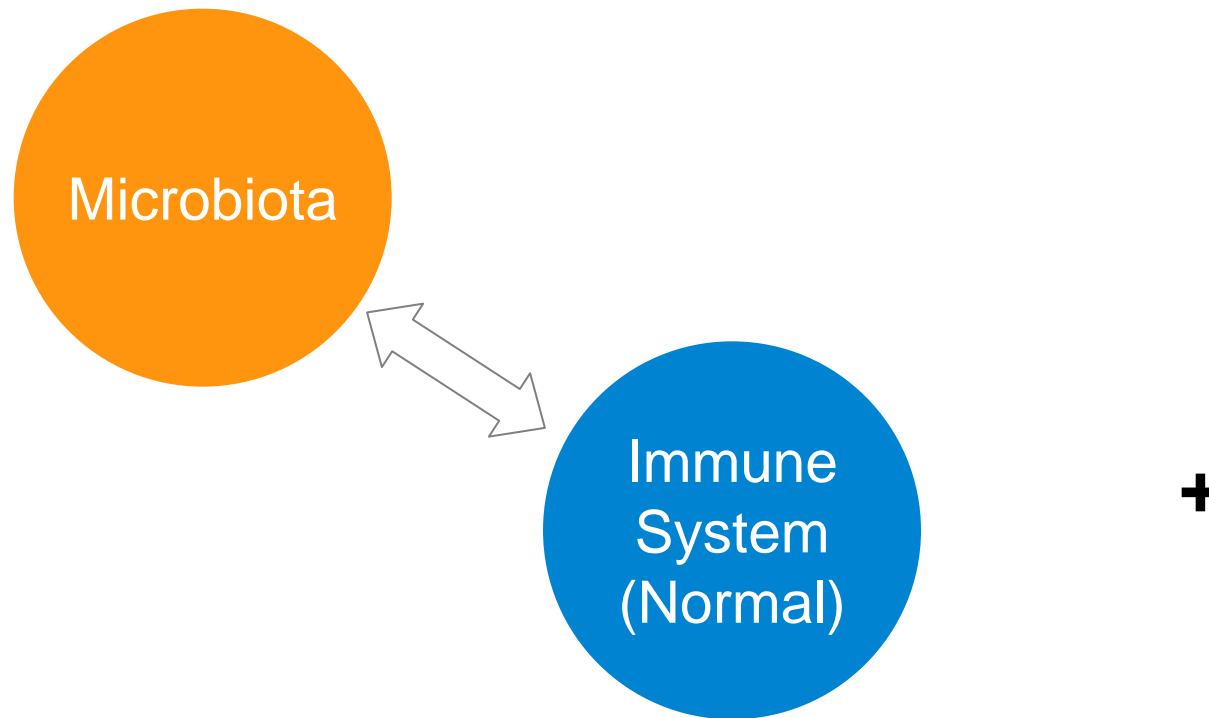
## Efecte del tractament d'adlimumab en micorbiota de pacients amb artritis reumatoide

**Table 3.** Bacterial loads of *Faecalibacterium prausnitzii*, *Escherichia coli* and Eubacteria in the biopsies at the two different times.

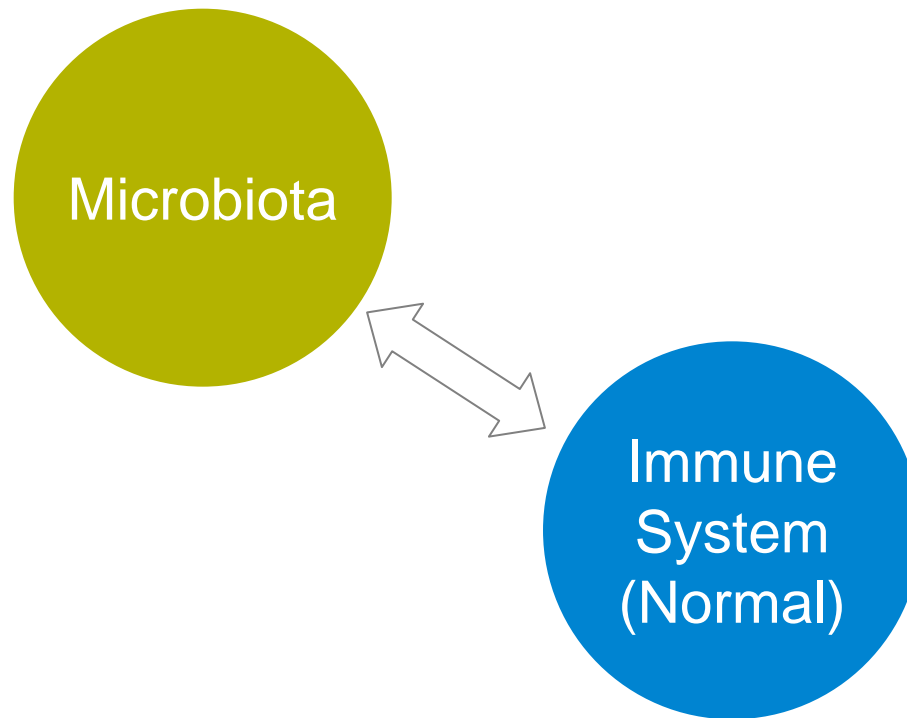
	Fpra		Eco		Eub	
	T0	T1	T0	T1	T0	T1
AR_05	1.000	0.877	1.149	0.776	1.000	1.267
AR_09	2.237	1.907	0.973	1.559	2.055	1.713
AR_10	1.000	1.855	2.574	0.550	1.000	1.988
AR_11	1.327		0.248		1.279	
AVG	1.391±0.585	1.546±0.580	1.236±0.974	0.962±0.529	1.333±0.499	1.656±0.364

- **Resposta immune** a la comunitat microbiana gastrointestinal
- **Composició** de la microbiota GI contribueix a la inflamació ( $\leftrightarrow$ )
- **Promotors de Treg** disminuïts; sobrecreixement de bactèries que indueixen poblacions cel·lulars **proinflamatòries Th17**

# Bacteria i sistema immune: Per tant, qui actua sobre qui?

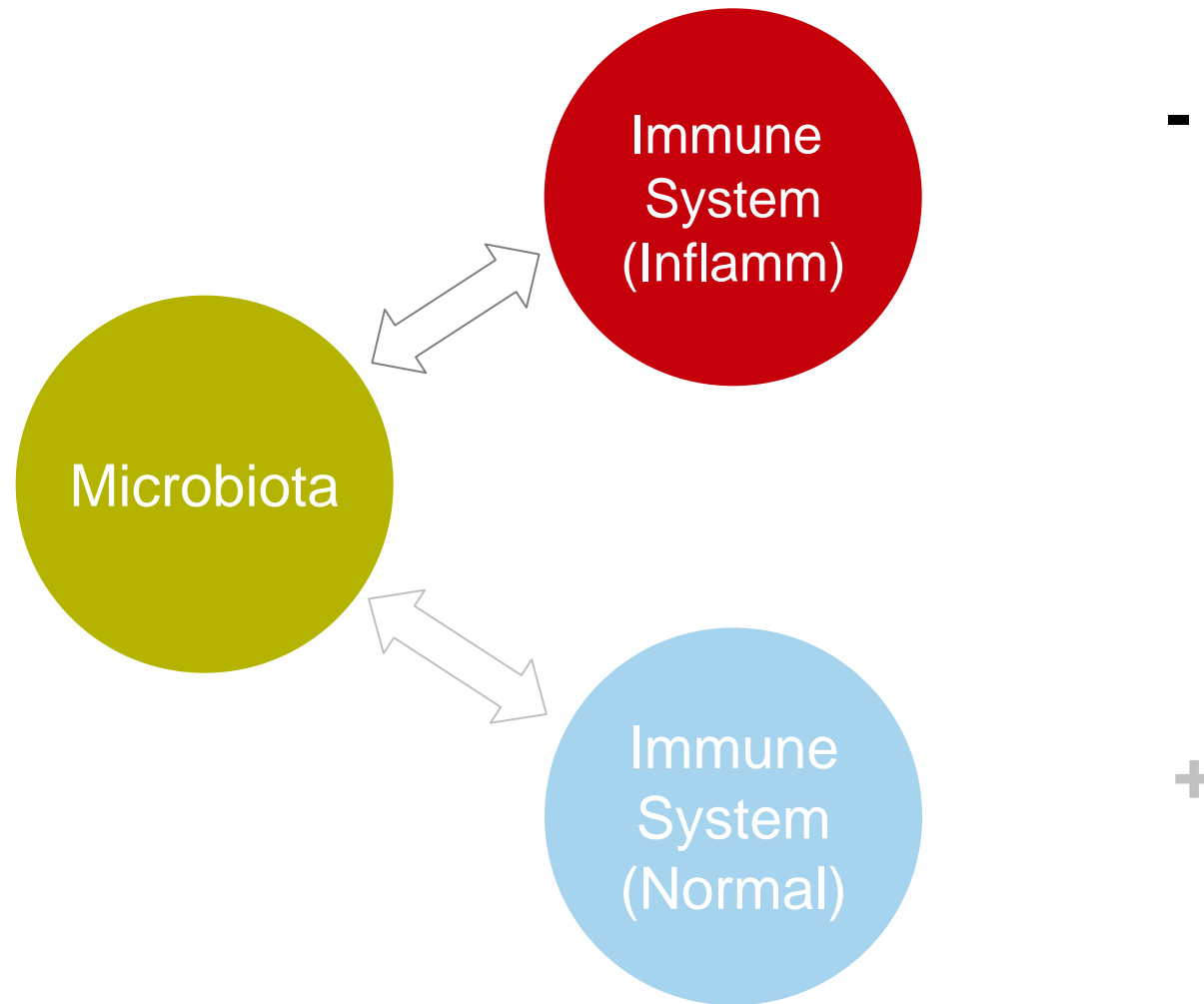


# Bacteria i sistema immune: Per tant, qui actua sobre qui?

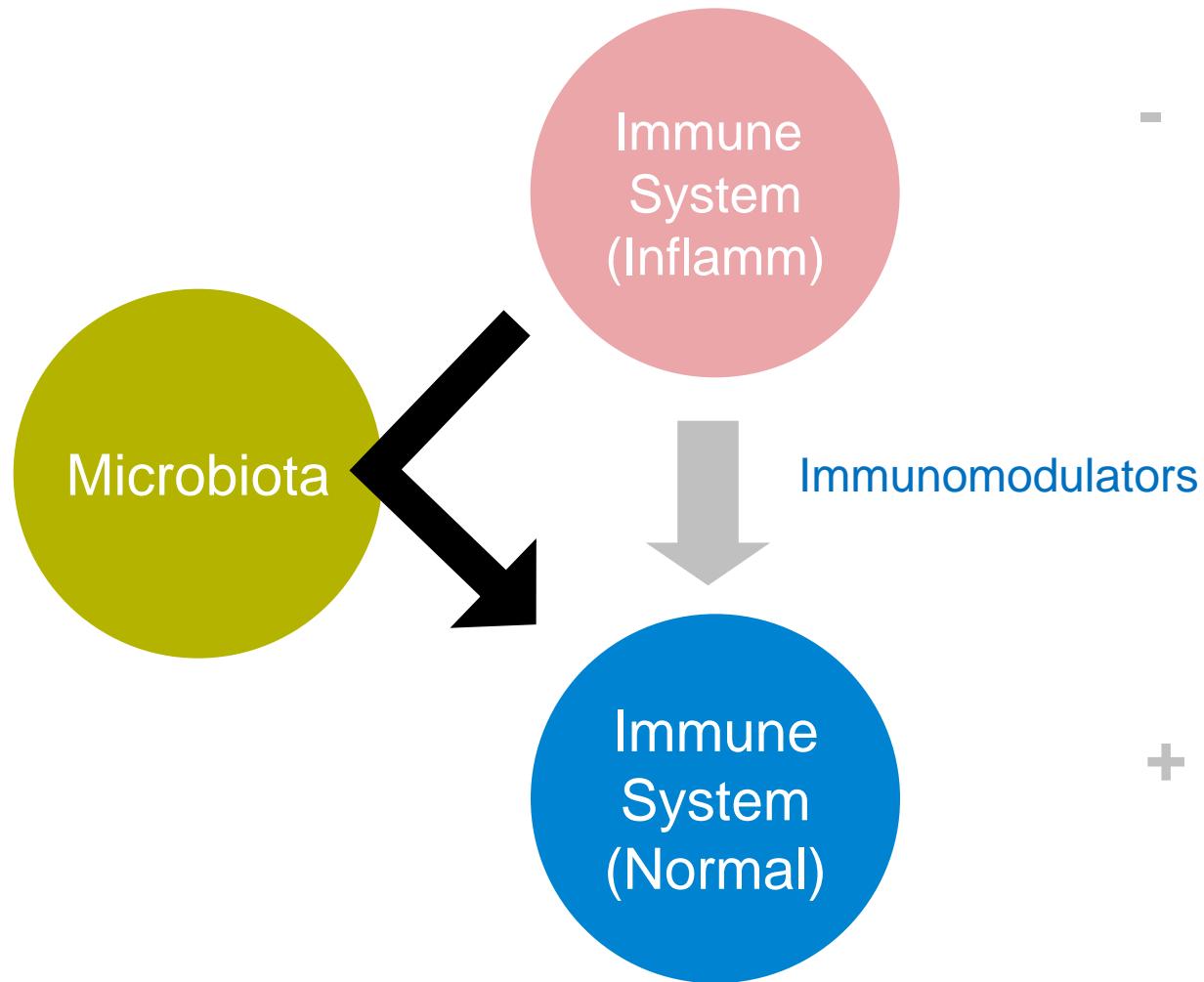




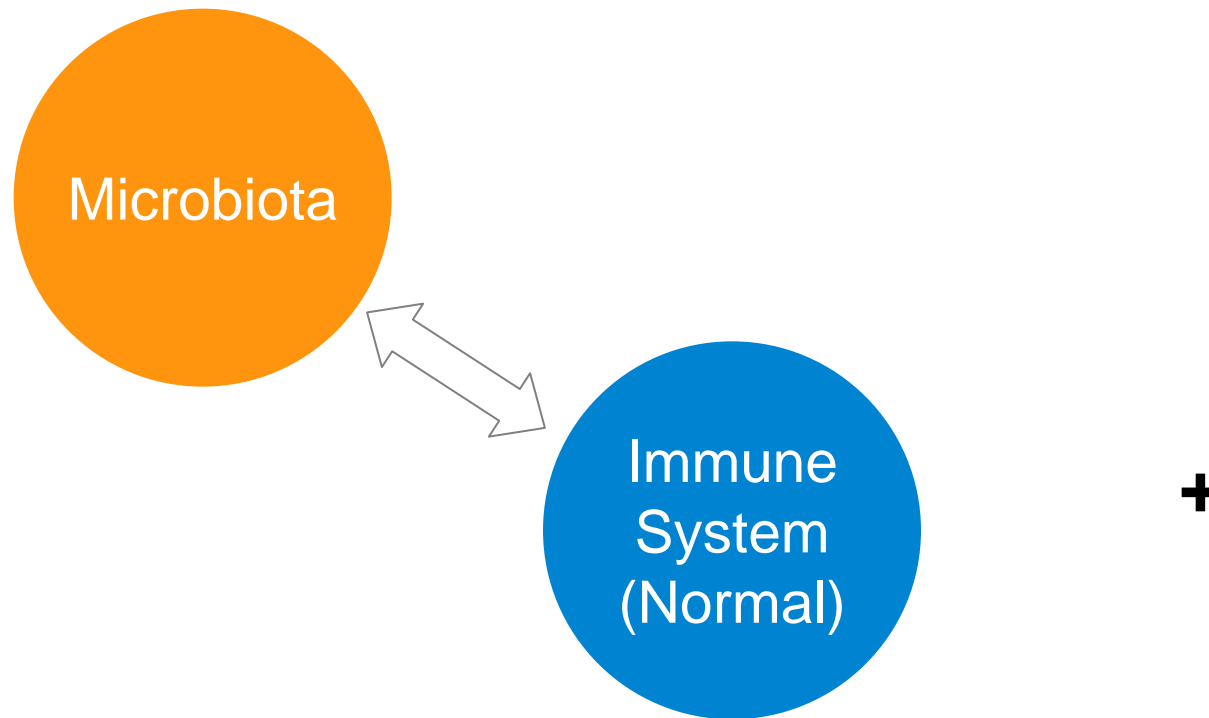
# Bacteria i sistema immune: Per tan,t qui actua sobre qui?



# Bacteria i sistema immune: Per tant, qui actua sobre qui?



# Bacteria i sistema immune: Per tant, qui actua sobre qui?



- Poc se sap sobre els efectes dels immunomoduladors a la microbiota
  - **Mesalazina**: trenca **biofilms bacterians**.
  - **Anti-TNF** : apunta cap a una recuperació de l'ecosistema com ho reveen els indicadors d'espècies
- Vies per les que la immunomodulació **restaura l'ecosistema**.
  - Incrementa la capa de moc (MTX)
  - Redueix el potencial redox
  - Promou creixement bacterià anaerobi (firmicutes)
- **Curació Mucosa**: Recuperació ecosistema
  - Ara: Curació mucosa = endoscòpica
  - Futur: Curació Mucosa = Restauració Microbiota



# Gràcies!

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