
**Istituto di Microbiologia**

***Università Cattolica del Sacro Cuore*, Roma**

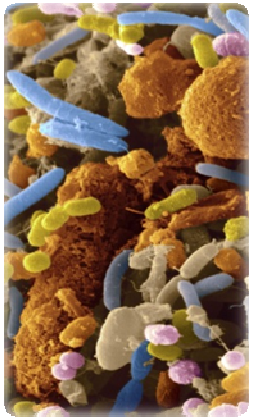

UNIVERSITÀ CATTOLICA del Sacro Cuore

# Il Microbiota:

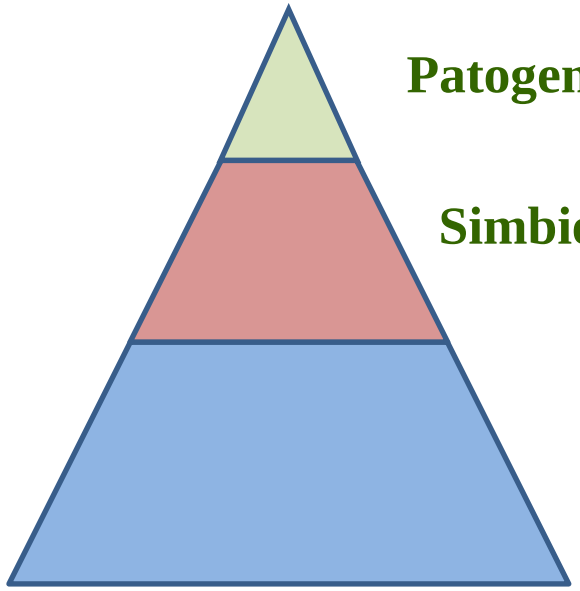
# questo sconosciuto


**Prof. Giovanni Fadda**

# Il microbiota
## (Bacteria,  Archea, Eukarya e Virus)



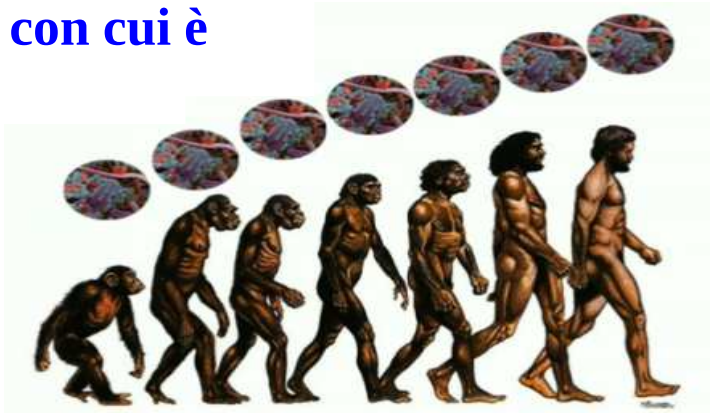
- **Storicamente i microbi sono stati associati con una connotazione negativa;**
- **$10^{30}$ batteri nel nostro pianeta;**
- **L'uomo vive in simbiosi con il microbiota, con cui è co-evoluto;**

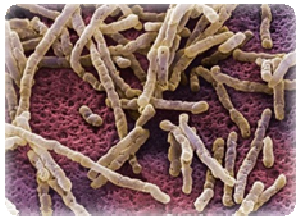


**Patogeni**

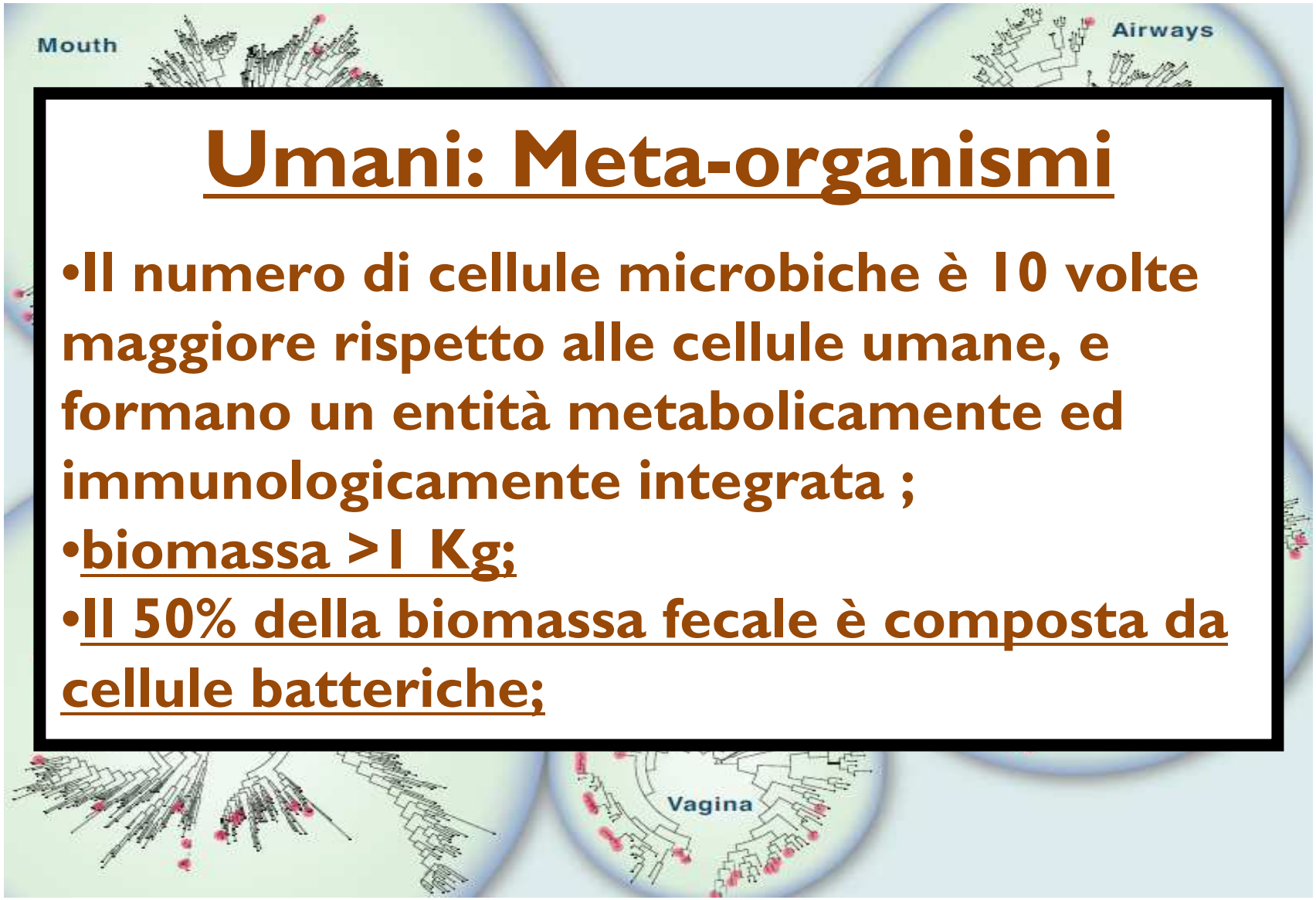
**Simbiosi - mutualistici**

**Commensali**





# Human Microbiota



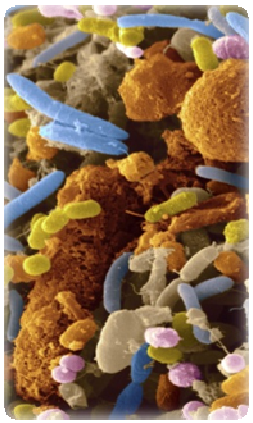

## Umani: Meta-organismi

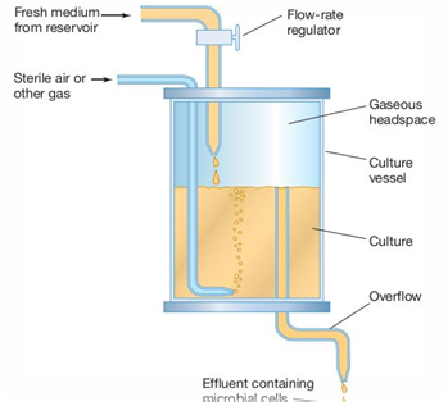
- Il numero di cellule microbiche è 10 volte maggiore rispetto alle cellule umane, e formano un entità metabolicamente ed immunologicamente integrata ;
- biomassa >1 Kg;
- Il 50% della biomassa fecale è composta da cellule batteriche;



Lee YKL, Science 2010

# Gut microbiota

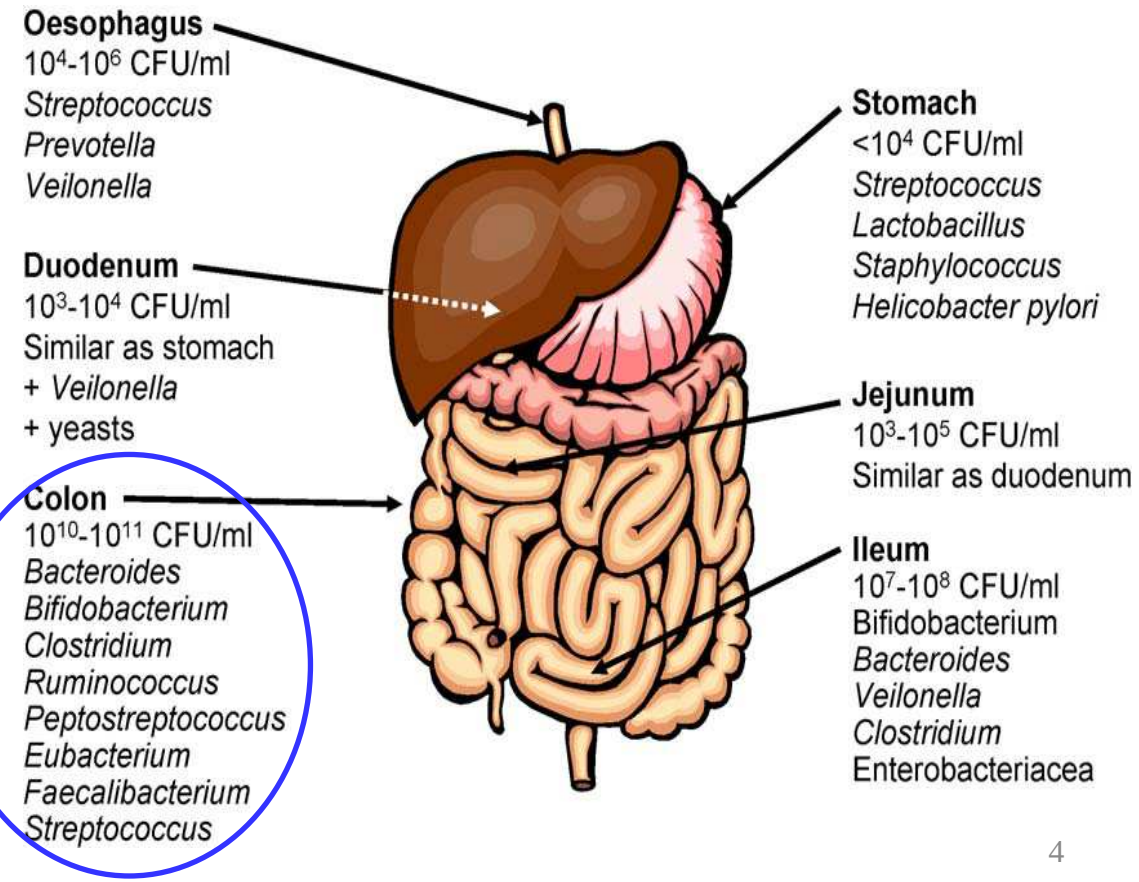




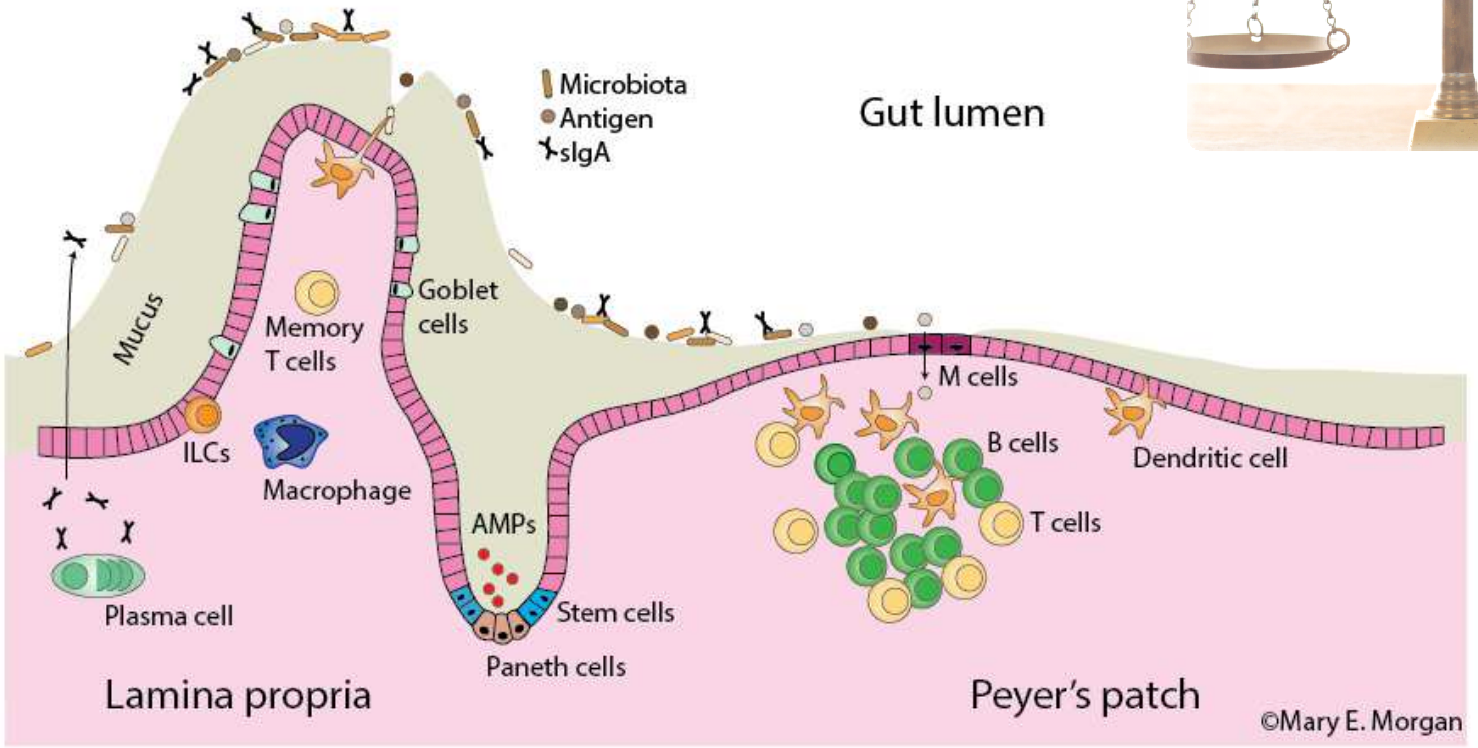

**BIOREATTORE**

- $10^{14}$ cellule batteriche;
- 1000 specie batteriche differenti.
- 90% cellule batteriche;
- 99% geni batterici (>3x$10^6$ mentre nell'uomo sono 23000);

**Specie microbiche presenti nel tratto gastrointestinale, con una densità ed diversità crescente**



# L’epitelio intestinale è l’interfaccia tra microbiota e tessuti dell’ospite.




**La composizione del microbiota è il risultato di un equilibrio tra l’abilità di sfruttare i nutrienti e la resistenza alle forze che tendono ad eliminarlo dall’intestino.**

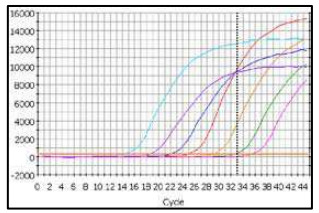
# Caratterizzazione del Gut Microbiota

**Tecniche colturali**

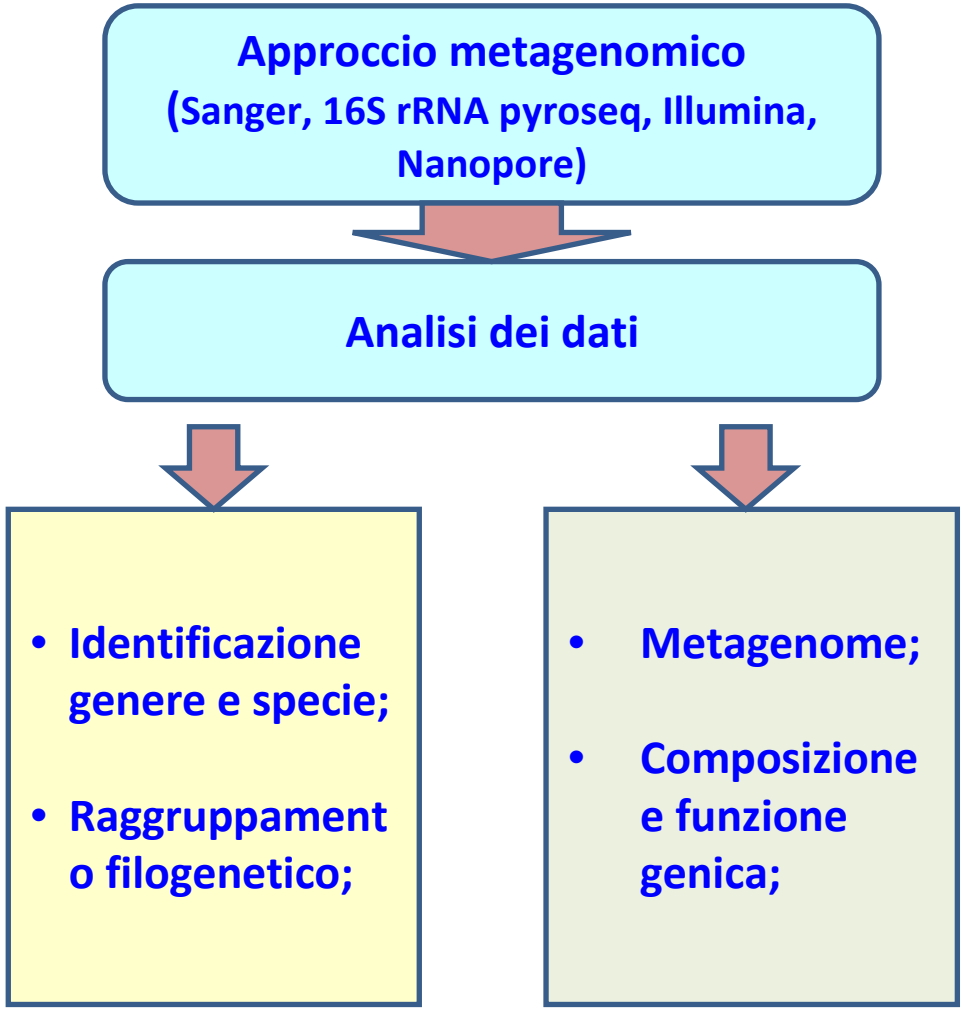
- **Non affidabile;**
- **Informazioni limitate;**

**Amplificazione DNA**

- **16S rRNA real time PCR;**
- **Batteri con coltivabili;**
- **Analisi (semi) quantitativa;**

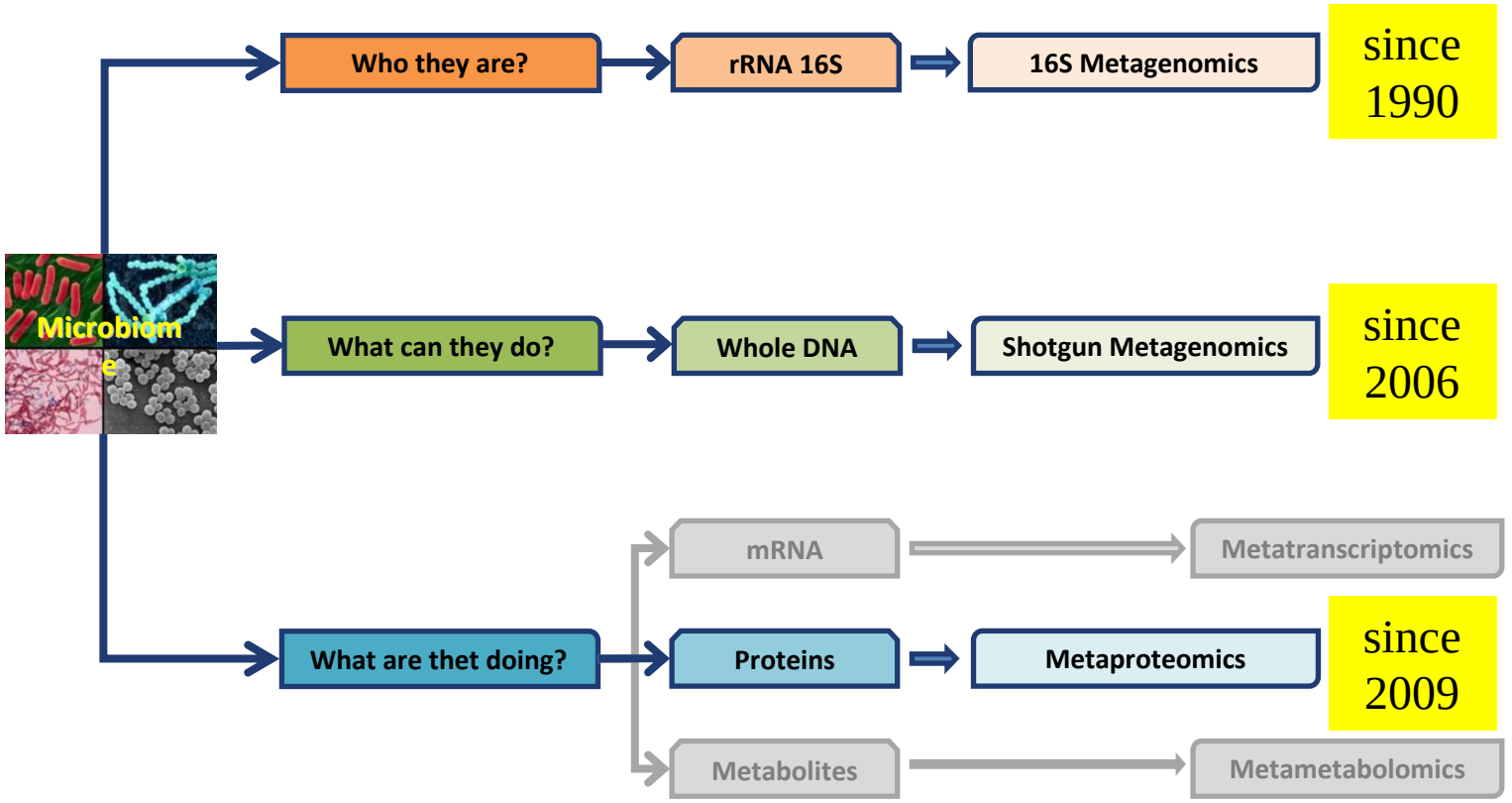


# Caratterizzazione del Gut Microbiota




- La quantità di informazione genetica già disponibile è impressionante e cresce a ritmo vertiginoso.
- Numerosissimi sono i genomi in corso di sequenziamento che saranno completati in pochi anni.
- La Metagenomica (detta anche genomica ambientale, ecogenomica o genomica delle comunità) è lo studio dei genomi recuperati da ambienti piuttosto che da singoli organismi coltivati: comunità intestinali, comunità marine, biofilm
- Meta-analisi: sintesi statistica di risultati provenienti da analisi separate
- Genomica: analisi completa del materiale genetico di un organismo

# Metodologie (OMICHE) utilizzate nello studio e caratterizzazione del microbioma




**WE NEED THEM ALL ! … BUT NOT ONLY THEM!**

**2014**

REVIEW ARTICLE

## The first 1000 cultured species of the human gastrointestinal microbiota

Mirjana Rajilić-Stojanović[1,2] & Willem M. de Vos[2,3]

[1]Department for Biotechnology and Biochemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia; [2]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands; and [3]Departments of Bacteriology and Immunology, and Veterinary Biosciences, University of Helsinki, Helsinki, Finland

**CULTUROMICS**

- Coltura con nutrienti speciali (rumine, feci, etc.);
- Identificazione con MALDI-TOF;

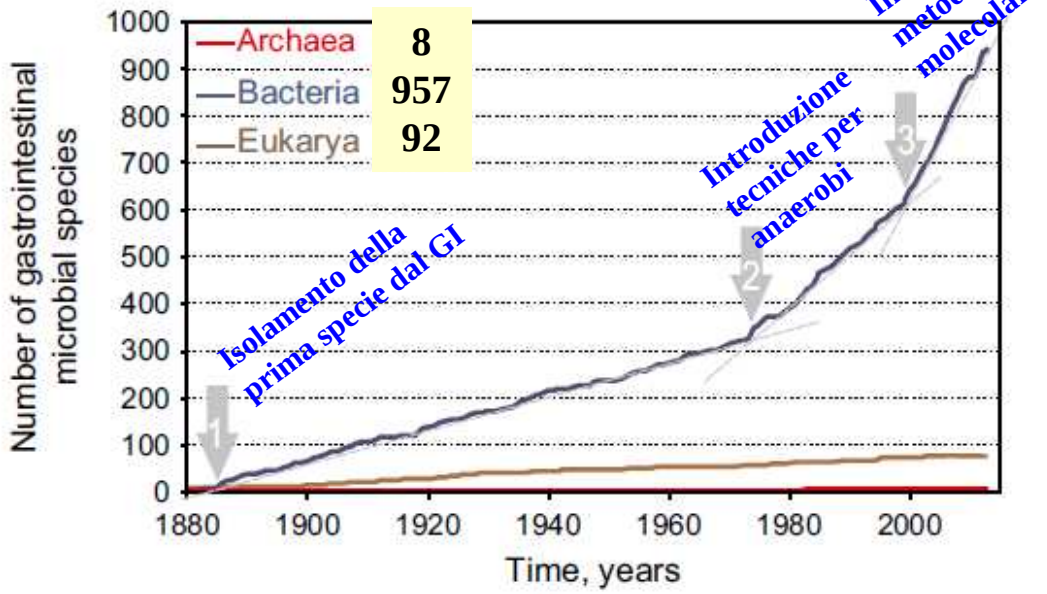



**Fig. 1.** Graphical representation of the cumulative number of cultured species from *Bacteria*, *Archaea* and *Eukarya* from the human gastrointestinal tract as a function of time. The arrows indicate the turning points of the gastrointestinal microbiota research: (1) Isolation of the first gastrointestinal bacterial species, (2) Introduction of strictly anaerobic techniques, and (3) Introduction of molecular techniques in the field of the gastrointestinal microbiota research.

REVIEW ARTICLE

# The first 1000 cultured species of the human gastrointestinal microbiota

Mirjana Rajilić-Stojanović[1,2] & Willem M. de Vos[2,3]

[1]Departm
[2]Laborato
Veterinary

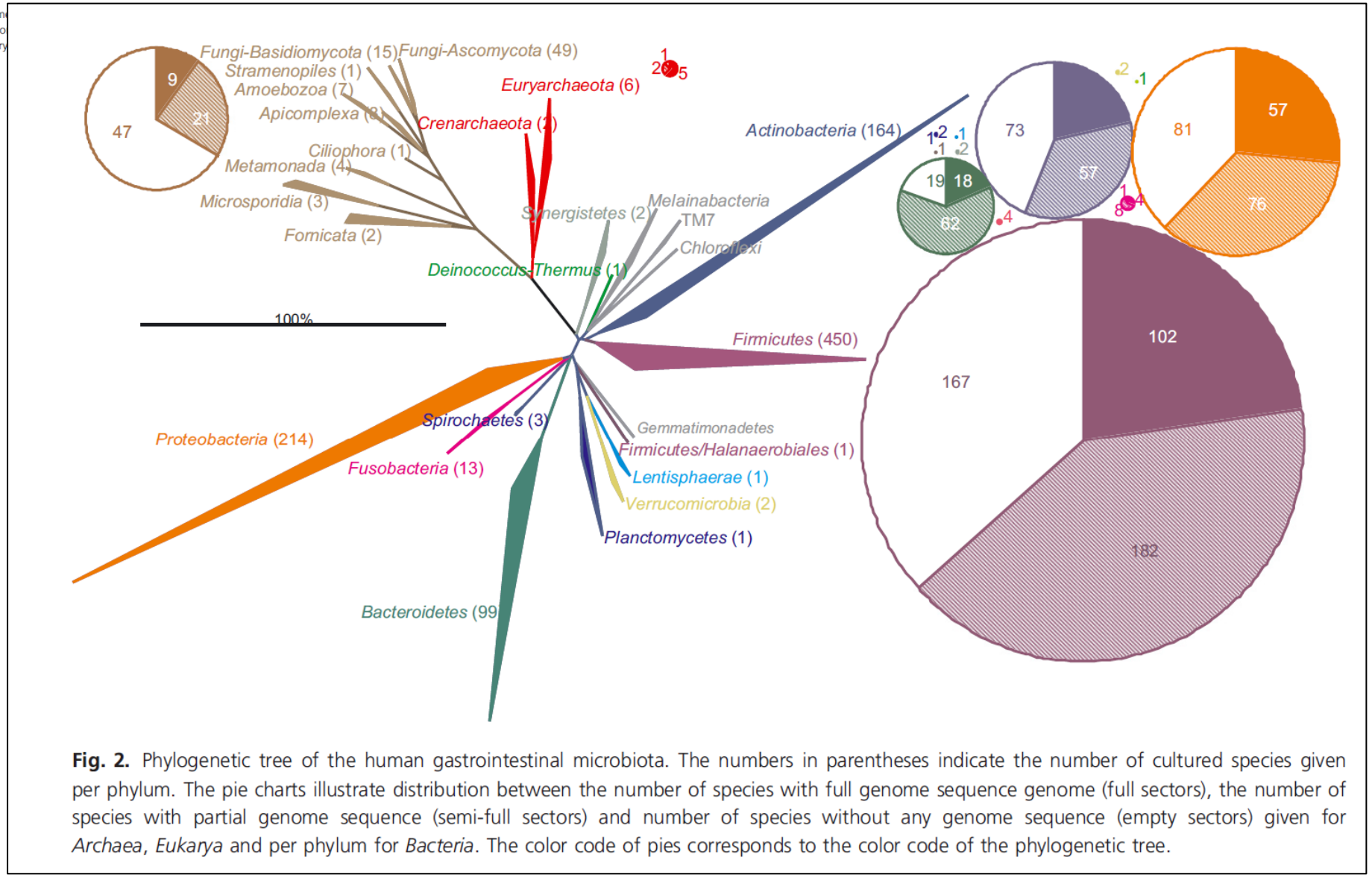



**Fig. 2.** Phylogenetic tree of the human gastrointestinal microbiota. The numbers in parentheses indicate the number of cultured species given per phylum. The pie charts illustrate distribution between the number of species with full genome sequence genome (full sectors), the number of species with partial genome sequence (semi-full sectors) and number of species without any genome sequence (empty sectors) given for *Archaea*, *Eukarya* and per phylum for *Bacteria*. The color code of pies corresponds to the color code of the phylogenetic tree.

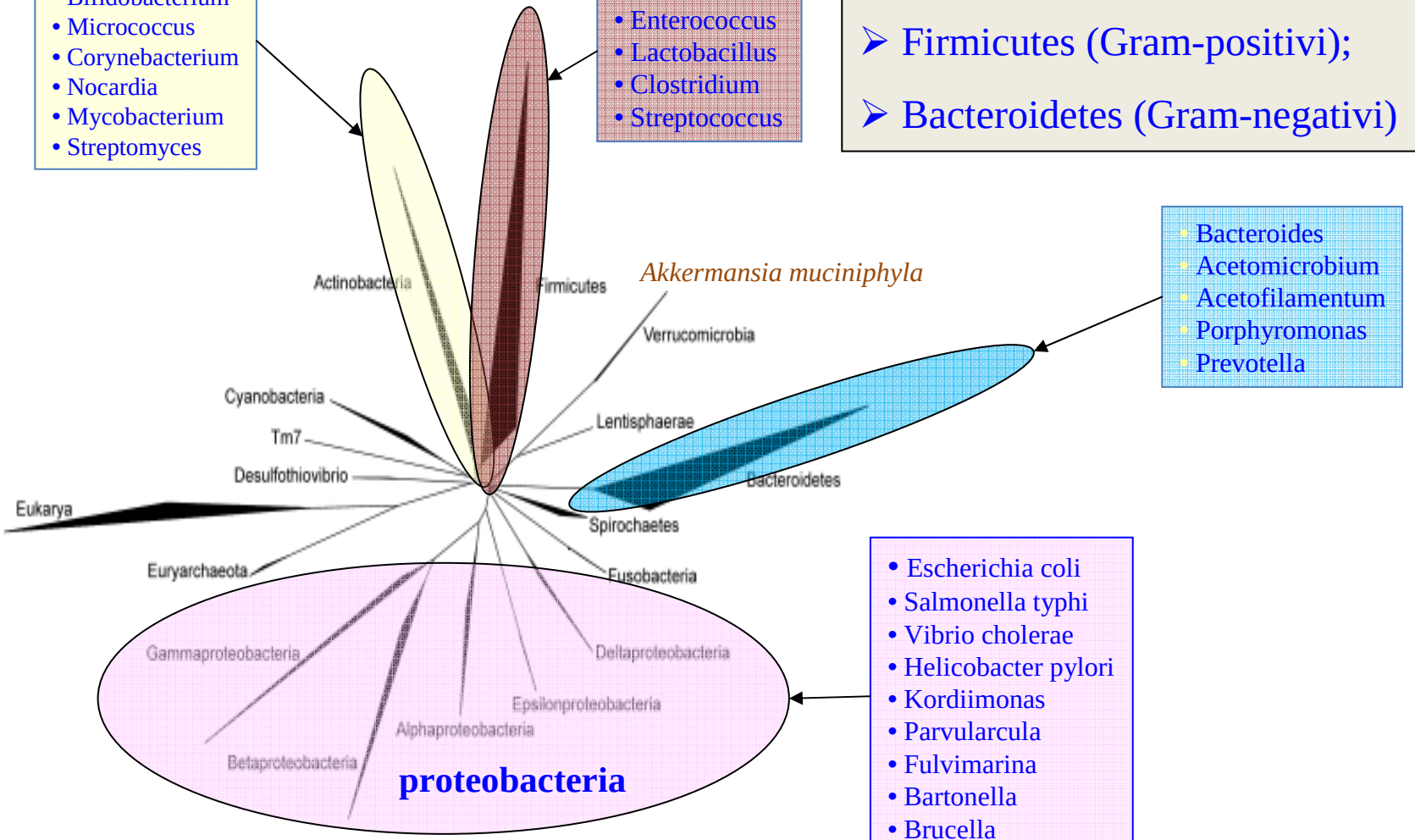
# La maggior parte delle specie batteriche presenti nell'intestino non possono essere coltivate nei comuni terreni di coltura

- Actinomyces
- Arthrobacter
- Bifidobacterium
- Micrococcus
- Corynebacterium
- Nocardia
- Mycobacterium
- Streptomyces

- Bacillus
- Enterococcus
- Lactobacillus
- Clostridium
- Streptococcus

2 phylum principali:

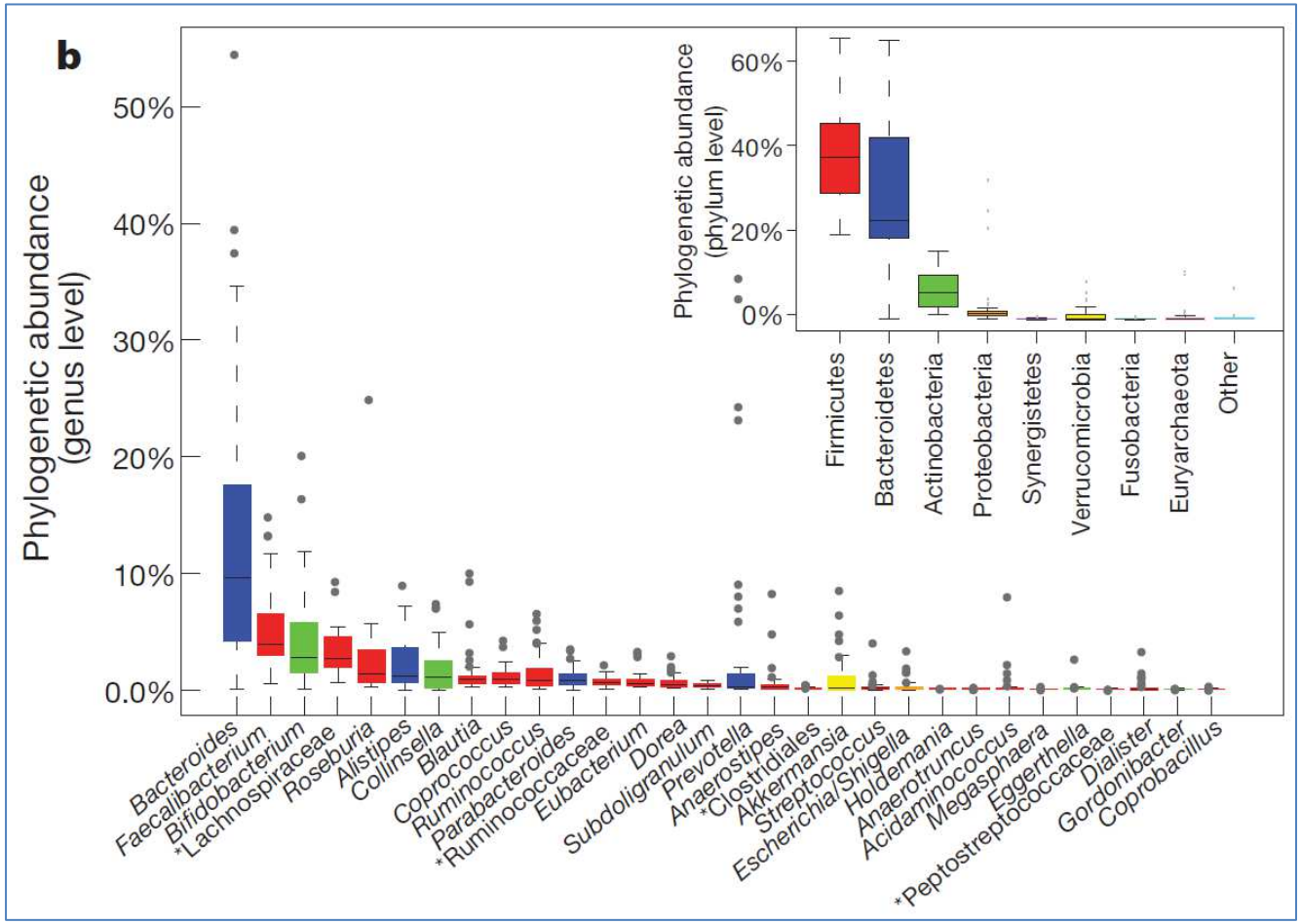
- Firmicutes (Gram-positivi);
- Bacteroidetes (Gram-negativi)




- Bacteroides
- Acetomicrobium
- Acetofilamentum
- Porphyromonas
- Prevotella

- Escherichia coli
- Salmonella typhi
- Vibrio cholerae
- Helicobacter pylori
- Kordiimonas
- Parvularcula
- Fulvimarina
- Bartonella
- Brucella

# Sequenziamento con metodo Sanger di 22 metagenomi di individui di Danesi, Francese, Italiani e Spagnoli.




**Profili funzionali e filogenetici del gut microbioma umano**

**Arumugam M. et al & MetaHIT consortium (2011) Enterotypes of the human gut microbiome. Nature 473, 174-180**

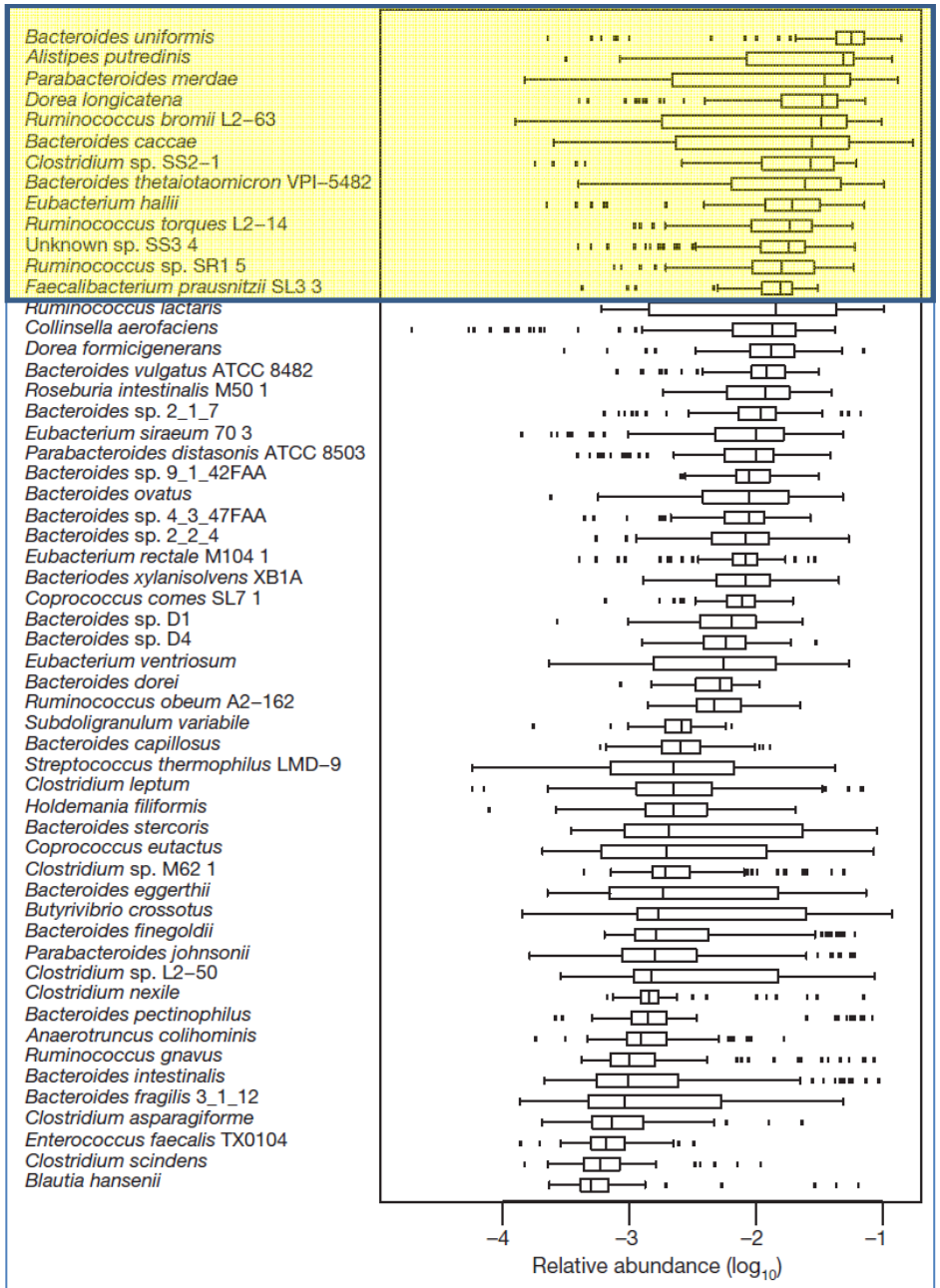
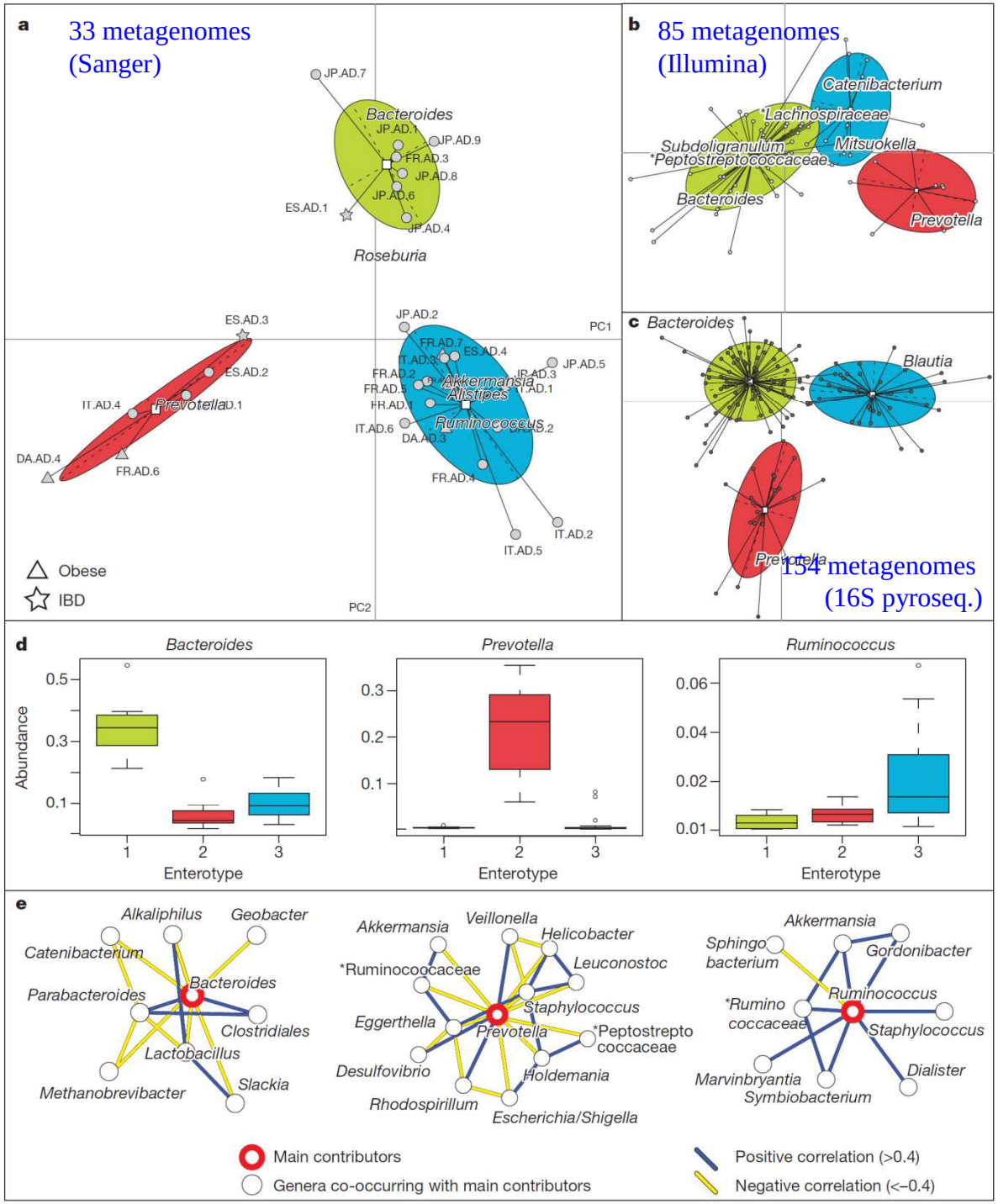



**Figure 3 | Relative abundance of 57 frequent microbial genomes among individuals of the cohort.** See Fig. 2c for definition of box and whisker plot. See Methods for computation.

- **Sequenziamento metagenomico con metodo Illumina;**
- **124 europei;**

**Qin J. Et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464, 59-65**




- **Ciascun Enterotipo è identificabile dalla predominanza di uno di tre generi : Bacteroides, Prevotella e Ruminococcus.**

- **Gli Enterotipi sono caratterizzati dalla presenza di gruppi di specie che contribuiscono a definire una determinata comunità microbica;**

**Enterotipo 1**

Fermentazione di carboidrati e proteine; elevato potenziale saccarolitico;

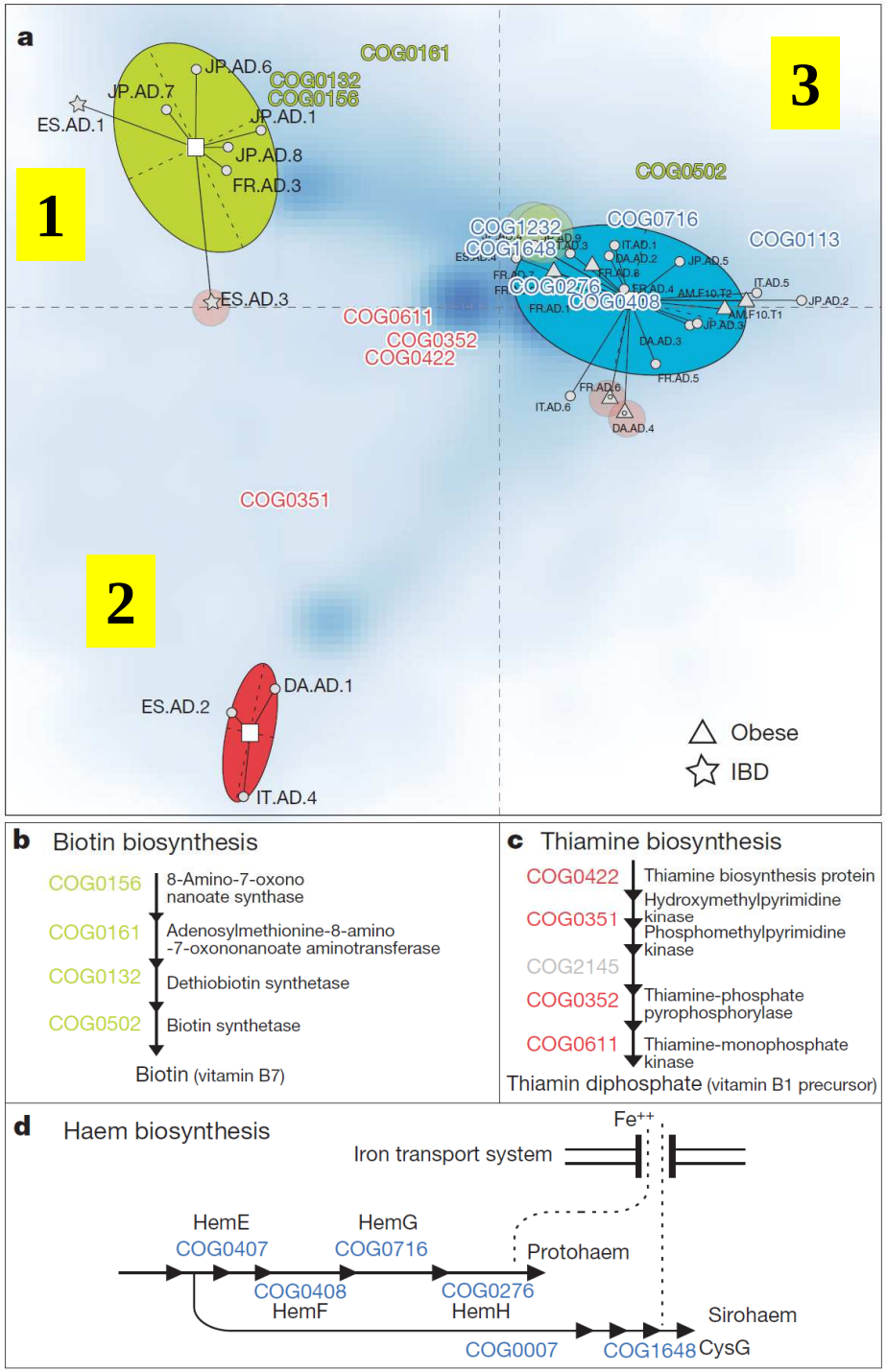
**Enterotipo 2**

Degradazione glicoproteine della mucina;

**Enterotipo 3**

Più frequente, comprende batteri capaci di degradare la mucina (*Akkermansia mucini*phila);

**Arumugam M. et al & MetaHIT consortium (2011) Enterotypes of the human gut microbiome. Nature 473, 174-180**




# Differenze funzionali tra enterotipi

**Le differenze funzionali e filogenetiche tra gli enterotipi riflettono combinazioni diverse tra *catene trofiche microbiche* con conseguente impatto e sinergia con l'ospite.**

**Arumugam M. et al & MetaHIT consortium (2011) Enterotypes of the human gut microbiome. Nature 473, 174-180**

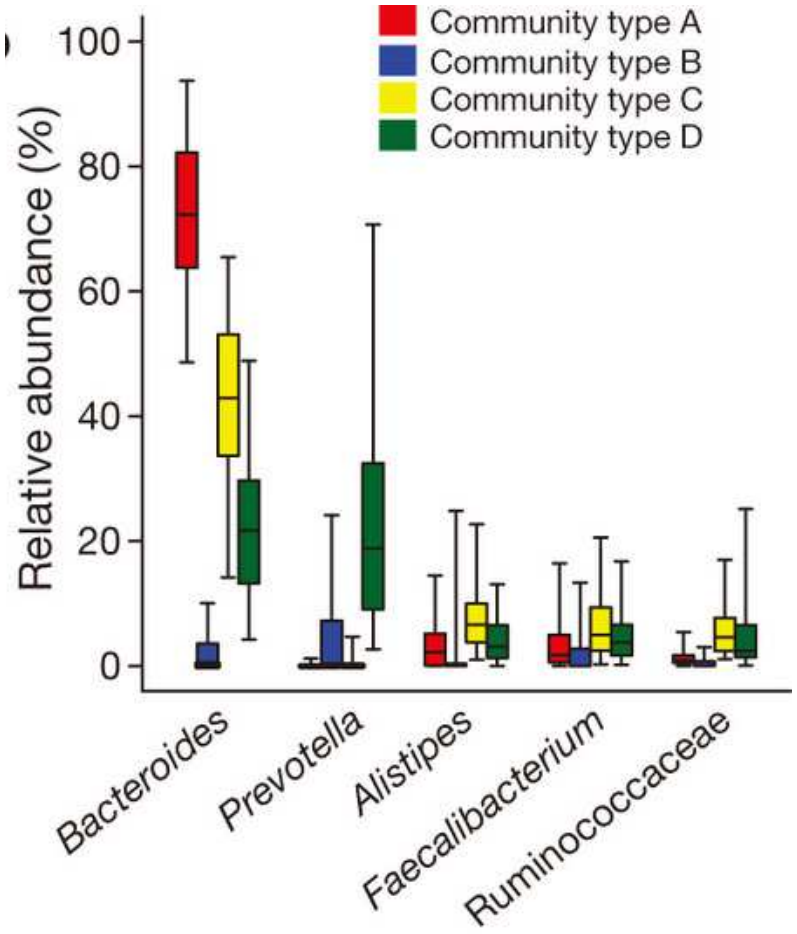
LETTER

15 MAY 2014 | VOL 509 | NATURE | 357

doi:10.1038/nature13178

# Dynamics and associations of microbial community types across the human body

Tao Ding[1] & Patrick D. Schloss[1]



ARTICLE

doi:10.1038/nature11234

# Structure, function and diversity of the healthy human microbiome

The Human Microbiome Project Consortium*

- **242 adulti (4788 campioni);**
- **I taxa microbici variano;**
- **I pathways metabolici rimangono stabili;**
- **Ridondanza funzionale;**

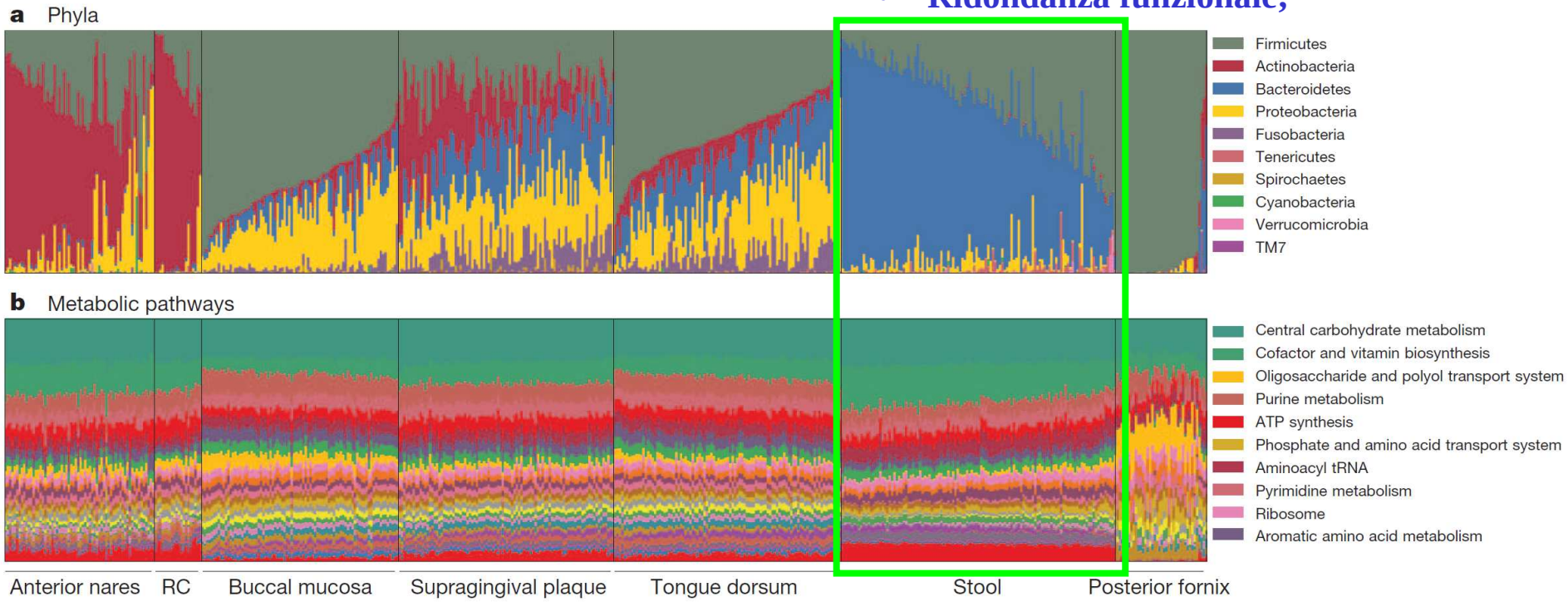



**Figure 2 | Carriage of microbial taxa varies while metabolic pathways remain stable within a healthy population.** **a**, **b**, Vertical bars represent microbiome samples by body habitat in the seven locations with both shotgun and 16S data; bars indicate relative abundances colored by microbial phyla from binned OTUs (**a**) and metabolic modules (**b**). Legend indicates most abundant phyla/pathways by average within one or more body habitats; RC, retroauricular crease. A plurality of most communities' memberships consists of a single dominant phylum (and often genus; see Supplementary Fig. 2), but this is universal neither to all body habitats nor to all individuals. Conversely, most metabolic pathways are evenly distributed and prevalent across both individuals and body habitats.

The HMP consortium. Structure, function and diversity of the healthy human microbiome. Nature 486 (June 2011)

# Come si forma il microbiota

## Alla nascita il corpo umano è sterile

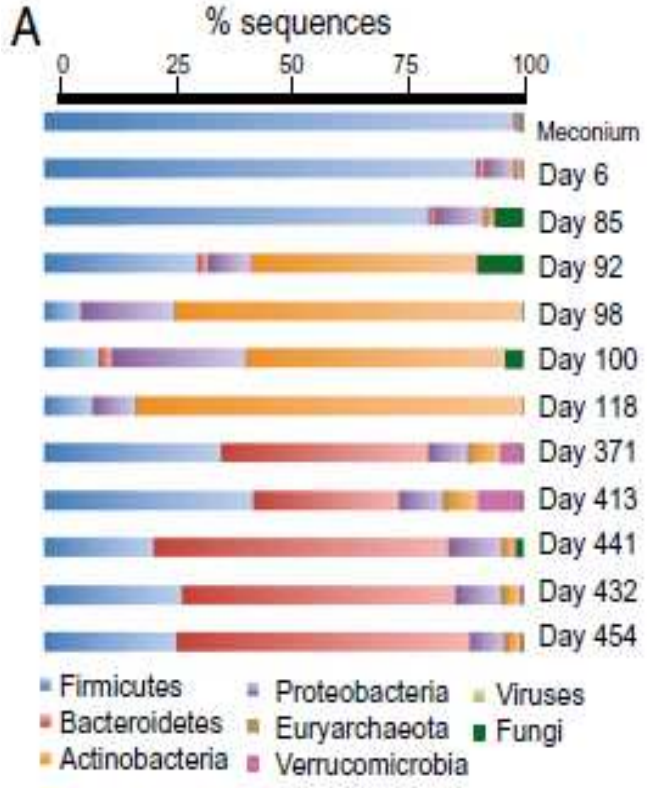
**Microbiota vaginale (madre)**

**Microbiota fecale (madre)**

**Microbiota della cute (genitori/parenti/babysitter)**

**Dieta**

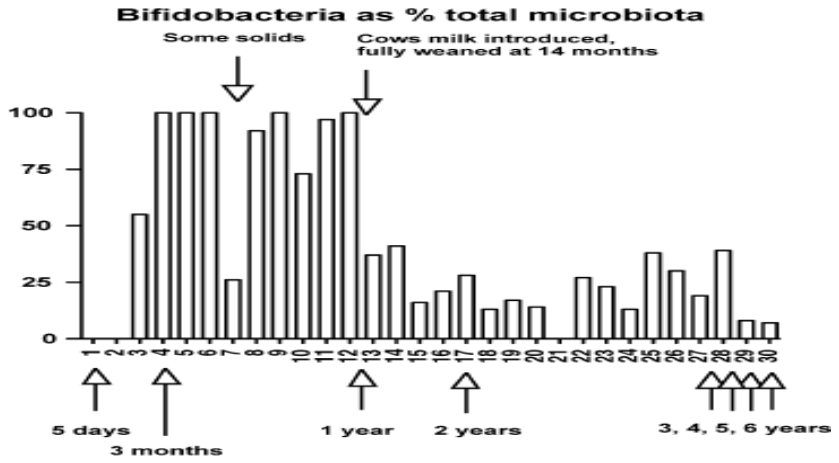
**Ambiente**




**CORE microbiota nativo (4-36 mesi di vita)**

# La composizione del Gut Microbiota è cruciale per l'Educazione Immunologica del neonato

- *Parto naturale/cesareo*
- *Ricovero in ospedale/casa*
- *Latte materno/artificiale*
- *Antibiotico terapia si/no*
- *Animali in casa*
- *Città/campagna*
- *Igiene poca/troppa?*
- *Numerosità familiare*




**Ad es.: La mancata esposizione dei bambini a specie del genere Bifidobacterium o alla loro eliminazione (ad es. antibioticoterapia) possono determinare uno squilibrio nella maturazione del sistema immunitario (deviazione immunologica).**

*Tannock, Semin Immunol 2007*

DIETA

ETA'

ETNIA

# Sommario

- **Ciascun individuo ha un microbiota «unico»:**

**Età, area geografica, dieta, etnia, etc.;**

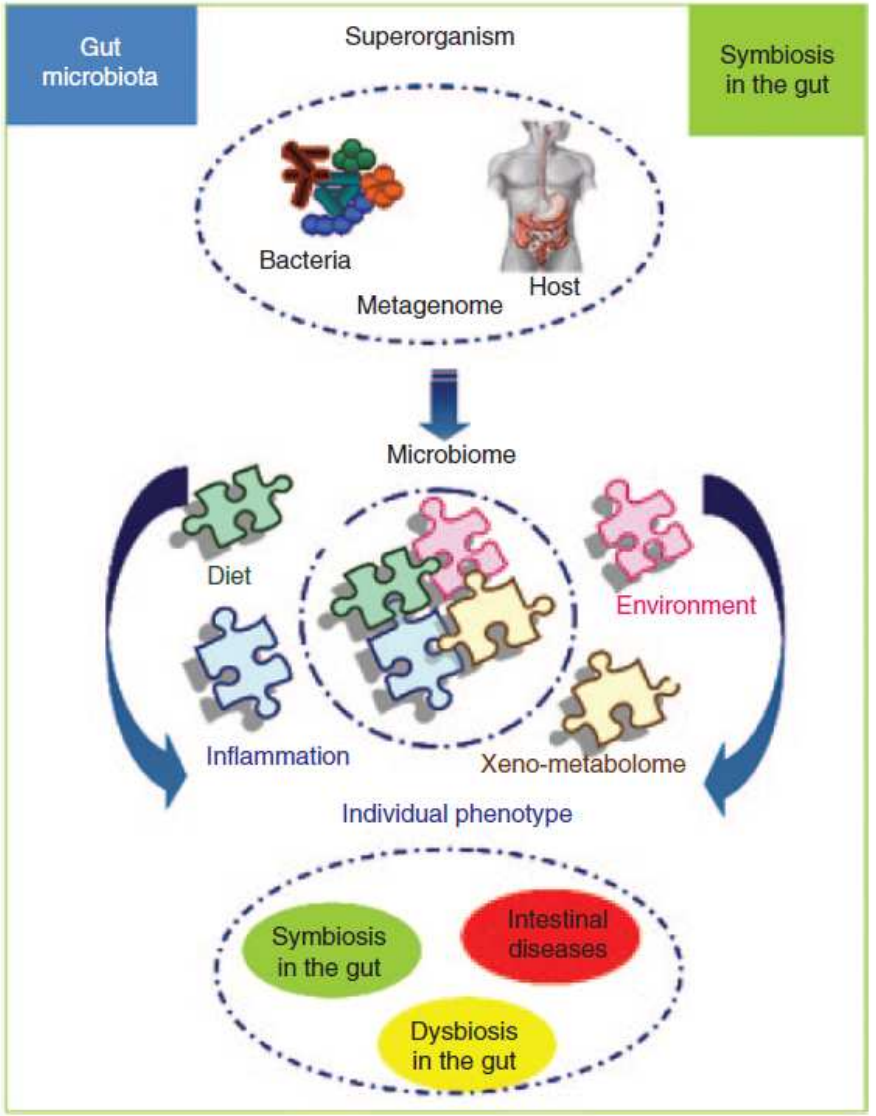
- **Numerosi eventi possono alterare in modo significativo il microbiota intestinale di un individuo:**

**Cambiamenti nella dieta, infezioni, antibiotico terapia, etc.;**

- **Il microbiota intestinale è «resiliente»:**

**Core microbiota/microbioma;**

- **Quando, per diverse ragioni, il microbiota non viene ripristinato può determinarsi una «disbiosi», le cui conseguenze possono essere molteplici**




*Putignani L. et al (2014) Pediatric Research 76(1)*

# Principali funzioni del microbiota intestinale

- **Metaboliche**

**Fermentazione di alimenti non-digeribili e del muco endogeno; recupero di energia sotto forma di acidi grassi a catena corta, produzione di vitamina K, assorbimento di ioni;**

- **Trofiche**

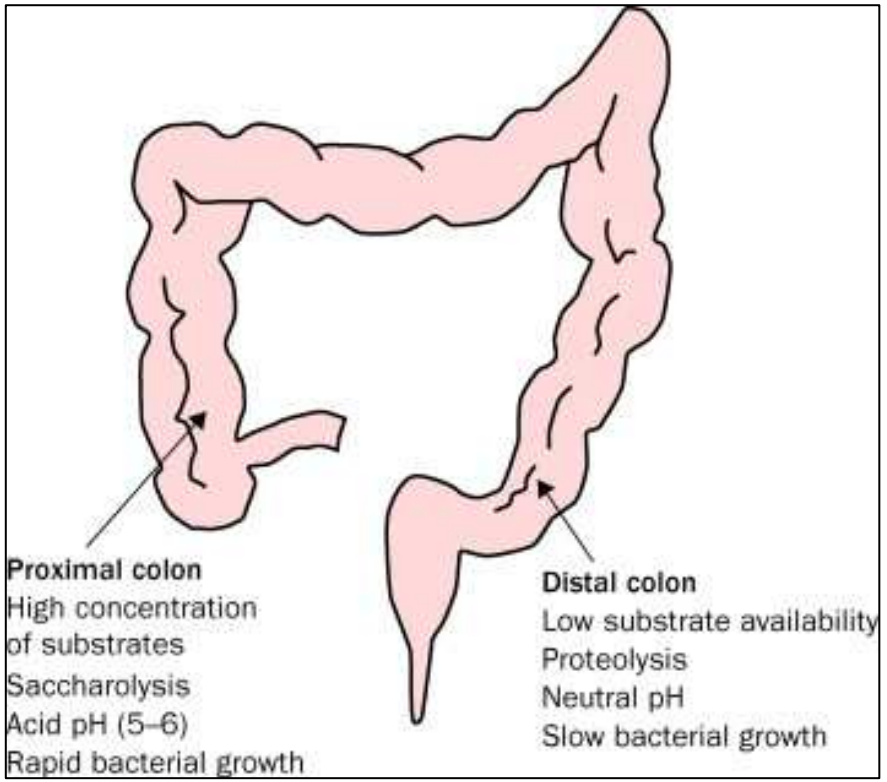
**Controllo della proliferazione e differenziamento dell'epitelio e sviluppo dei microvilli;**

- **Immunologico**

**Sviluppo e omeostasi del sistema immunitario;**

- **Protettivo**

**Protezione dai patogeni (effetto barriera);**

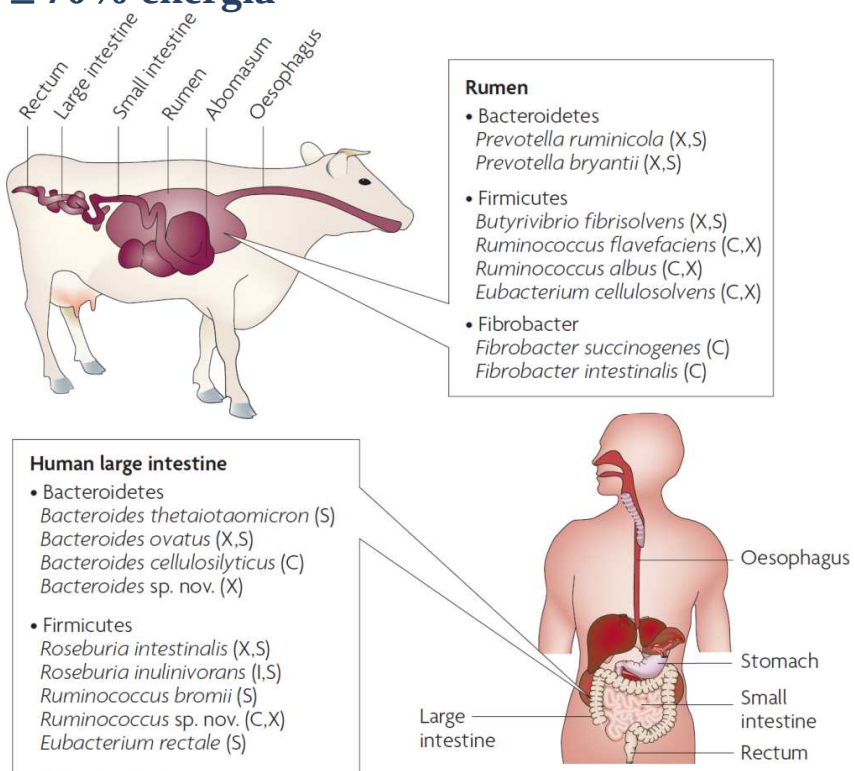



**Guarner F. and Malagelada JR (2003) Lancet 361**

# Principali funzioni del microbiota intestinale

- **Digestione dei carboidrati complessi.**
- **Produzione di vitamine e acidi grassi;**
- Maturazione del sistema immunitario intestinale;
- Regolazione della mobilità intestinale;
- Barriera contro la proliferazione dei patogeni;

**≅ 70% energia**




**≅ 10% energia**

**Acidi grassi a catena corta, butirrato, acetato e propionato;**

Science
AAAS

**Host-Gut Microbiota Metabolic Interactions**
Jeremy K. Nicholson *et al.*
*Science* **336**, 1262 (2012);
DOI: 10.1126/science.1223813


**Table 1.** Gut bacteria and the metabolites they contribute.

**Table 1.** Gut bacteria and the metabolites they contribute.

| Metabolites | Related bacteria | Potential biological functions | References |
|---|---|---|---|
| Short-chain fatty acids: acetate, propionate, butyrate, isobutyrate, 2-methylpropionate, valerate, isovalerate, hexanoate | Clostridial clusters IV and XIVa of *Firmicutes*, including species of *Eubacterium*, *Roseburia*, *Faecalibacterium*, and *Coprococcus* | Decreased colonic pH, inhibit the growth of pathogens; stimulate water and sodium absorption; participate in cholesterol synthesis; provide energy to the colonic epithelial cells, implicated in human obesity, insulin resistance and type 2 diabetes, colorectal cancer. | (*13*, *24*, *57*) |
| Bile acids: cholate, hyocholate, deoxycholate, chenodeoxycholate, α-muricholate, β-muricholate, ω-muricholate, taurocholate, glycocholate, taurochenoxycholate, glycochenodeoxycholate, taurocholate, tauro-α-muricholate, tauro-β-muricholate, lithocholate, ursodeoxycholate, hyodeoxycholate, glycodeoxylcholate, taurohyocholate, taurodeoxylcholate | *Lactobacillus*, *Bifidobacteria*, *Enterobacter*, *Bacteroides*, *Clostridium* | Absorb dietary fats and lipid-soluble vitamins, facilitate lipid absorption, maintain intestinal barrier function, signal systemic endocrine functions to regulate triglycerides, cholesterol, glucose and energy homeostasis. | (*30*, *31*, *33*) |
| Choline metabolites: methylamine, dimethylamine, trimethylamine, trimethylamine-*N*-oxide, dimethylglycine, betaine | *Faecalibacterium prausnitzii*, *Bifidobacterium* | Modulate lipid metabolism and glucose homeostasis. Involved in nonalcoholic fatty liver disease, dietary induced obesity, diabetes, and cardiovascular disease. | (*29*, *58*) |
| Polyamines: putrescine, cadaverine, spermidine, spermine | *Campylobacter jejuni*, *Clostridium saccharolyticum* | Exert genotoxic effects on the host, anti-inflammatory and antitumoral effects. Potential tumor markers. | (*66*, *67*) |
| Lipids: conjugated fatty acids, LPS, peptidoglycan, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides | *Bifidobacterium*, *Roseburia*, *Lactobacillus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Clostridium* | Impact intestinal permeability, activate intestine-brain-liver neural axis to regulate glucose homeostasis; LPS induces chronic systemic inflammation; conjugated fatty acids improve hyperinsulinemia, enhance the immune system and alter lipoprotein profiles. Cholesterol is the basis for sterol and bile acid production. | (*42*, *68*) |
| Others: D-lactate, formate, methanol, ethanol, succinate, lysine, glucose, urea, α-ketoisovalerate, creatine, creatinine, endocannabinoids, 2-arachidonoylglycerol (2-AG), *N*-arachidonoylethanolamide, LPS, etc. | *Bacteroides*, *Pseudobutyrivibrio*, *Ruminococcus*, *Faecalibacterium*, *Subdoligranulum*, *Bifidobacterium*, *Atopobium*, *Firmicutes*, *Lactobacillus* | Direct or indirect synthesis or utilization of compounds or modulation of linked pathways including endocannabinoid system. | (*43*, *60*, *69*) |

**Host-Gut Microbiota Metabolic Interactions**
Jeremy K. Nicholson *et al.*
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**Table 1.** Gut bacteria and the metabolites they contribute.

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| Metabolites | Related bacteria | Potential biological functions | References |
|---|---|---|---|
| Phenolic, benzoyl, and phenyl derivatives: benzoic acid, hippuric acid, 2-hydroxyhippuric acid, 2-hydroxybenzoic acid, 3-hydroxyhippuric acid, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 3-hydroxyphenylpropionate, 4-hydroxyphenylpropionate, 3-hydroxycinnamate, 4-methylphenol, tyrosine, phenylalanine, 4-cresol, 4-cresyl sulfate, 4-cresyl glucuronide, 4-hydroxyphenylacetate, 3,4-dihydroxyphenylacetate, phenylacetylglycine, phenylacetylglutamine, phenylacetylglycine, phenylacetate, phenylpropionate, phenylpropionylglycine, cinnamoylglycine | *Clostridium difficile*, *F. prausnitzii*, *Bifidobacterium*, *Subdoligranulum*, *Lactobacillus* | Detoxification of xenobiotics; indicate gut microbial composition and activity; utilize polyphenols. Urinary hippuric acid may be a biomarker of hypertension and obesity in humans. Urinary 4-hydroxyphenylacetate, 4-cresol, and phenylacetate are elevated in colorectal cancer. Urinary 4-cresyl sulfate is elevated in children with severe autism. | (*59*, *60*) |
| Indole derivatives: *N*-acetyltryptophan, indoleacetate, indoleacetylglycine (IAG), indole, indoxyl sulfate, indole-3-propionate, melatonin, melatonin 6-sulfate, serotonin, 5-hydroxyindole | *Clostridium sporogenes*, *E. coli* | Protect against stress-induced lesions in the GI tract; modulate expression of proinflammatory genes, increase expression of anti-inflammatory genes, strengthen epithelial cell barrier properties. Implicated in GI pathologies, brain-gut axis, and a few neurological conditions. | (*61*–*63*) |
| Vitamins: vitamin K, vitamin B12, biotin, folate, thiamine, riboflavin, pyridoxine | *Bifidobacterium* | Provide complementary endogenous sources of vitamins, strengthen immune function, exert epigenetic effects to regulate cell proliferation. | (*64*, *65*) |
| Others: D-lactate, formate, methanol, ethanol, succinate, lysine, glucose, urea, α-ketoisovalerate, creatine, creatinine, endocannabinoids, 2-arachidonoylglycerol (2-AG), *N*-arachidonoylethanolamide, LPS, etc. | *Bacteroides*, *Pseudobutyrivibrio*, *Ruminococcus*, *Faecalibacterium*, *Subdoligranulum*, *Bifidobacterium*, *Atopobium*, *Firmicutes*, *Lactobacillus* | is the basis for sterol and bile acid production. Direct or indirect synthesis or utilization of compounds or modulation of linked pathways including endocannabinoid system. | (*43*, *60*, *69*) |

**Host-Gut Microbiota Metabolic Interactions**
Jeremy K. Nicholson *et al.*
*Science* **336**, 1262 (2012);
DOI: 10.1126/science.1223813


**Table 1.** Gut bacteria and the metabolites they contribute.

**Table 1.** Gut bacteria and the metabolites they contribute.

| Metabolites | Related bacteria | Potential biological functions | References |
|---|---|---|---|
| Polyamines: putrescine, cadaverine, spermidine, spermine | *Campylobacter jejuni*, *Clostridium saccharolyticum* | Exert genotoxic effects on the host, anti-inflammatory and antitumoral effects. Potential tumor markers. | (*66*, *67*) |
| Lipids: conjugated fatty acids, LPS, peptidoglycan, acylglycerols, sphingomyelin, cholesterol, phosphatidylcholines, phosphoethanolamines, triglycerides | *Bifidobacterium*, *Roseburia*, *Lactobacillus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Clostridium* | Impact intestinal permeability, activate intestine-brain-liver neural axis to regulate glucose homeostasis; LPS induces chronic systemic inflammation; conjugated fatty acids improve hyperinsulinemia, enhance the immune system and alter lipoprotein profiles. Cholesterol is the basis for sterol and bile acid production | (*42*, *68*) |
| Others: D-lactate, formate, methanol, ethanol, succinate, lysine, glucose, urea, α-ketoisovalerate, creatine, creatinine, endocannabinoids, 2-arachidonoylglycerol (2-AG), *N*-arachidonoylethanolamide, LPS, etc. | *Bacteroides*, *Pseudobutyrivibrio*, *Ruminococcus*, *Faecalibacterium*, *Subdoligranulum*, *Bifidobacterium*, *Atopobium*, *Firmicutes*, *Lactobacillus* | Direct or indirect synthesis or utilization of compounds or modulation of linked pathways including endocannabinoid system. | (*43*, *60*, *69*) |


| | | | |
|---|---|---|---|
| | | properties. Implicated in GI pathologies, brain-gut axis, and a few neurological conditions. | |
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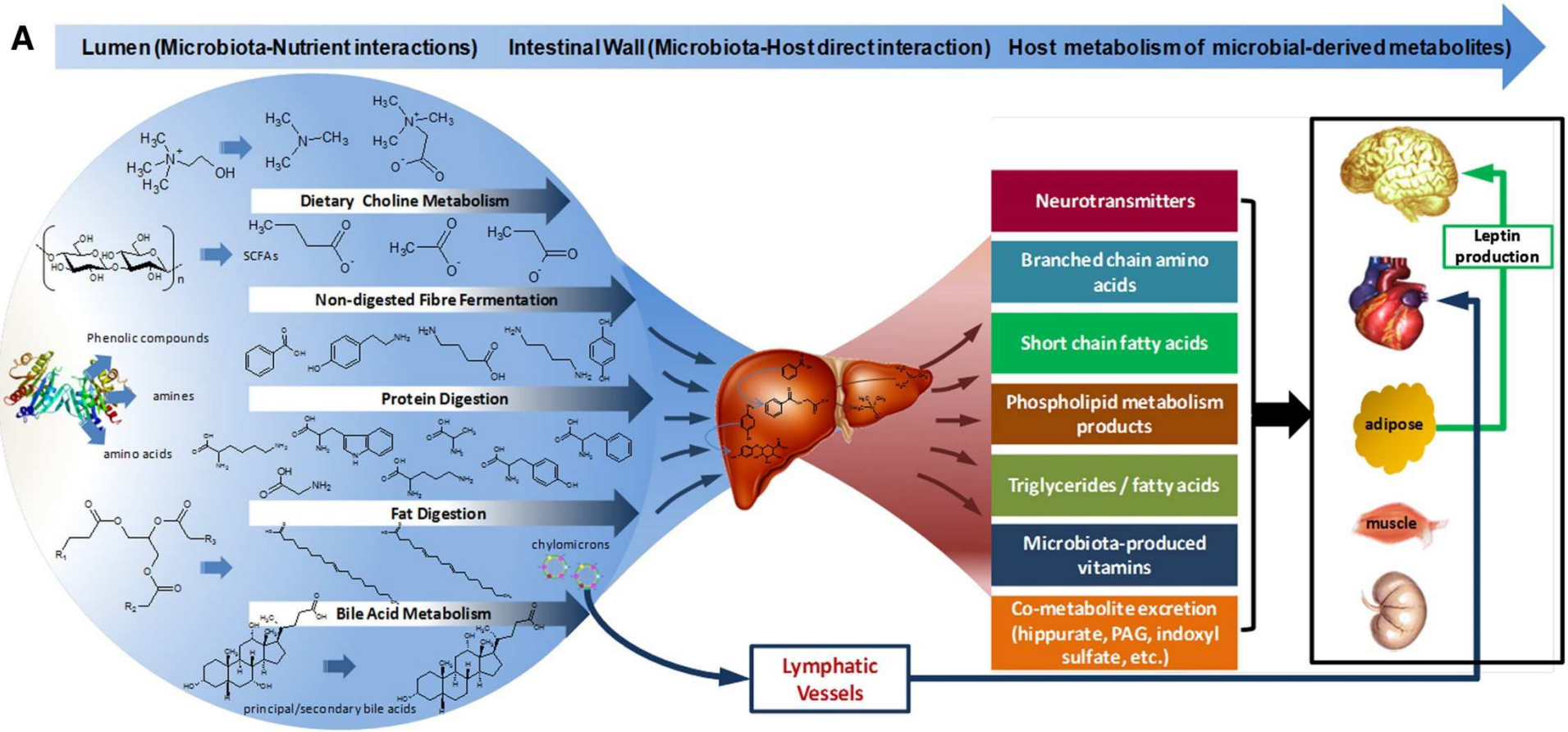
# Microbiota e metabolismo




**Fino ad 1/3 delle piccole molecole presenti nel sangue possono derivare dal gut microbiota.**

**Effetto positivo: attività anti-infiammatoria, anti-ossidante, antidolorifica, vitamine, sorgenti di energia;**

**Effetti tossici: cito-, geno-, immuno- tossine. Ad es LPS induce infiammazione che poi può portare ad obesità e insulino-resistenza;**

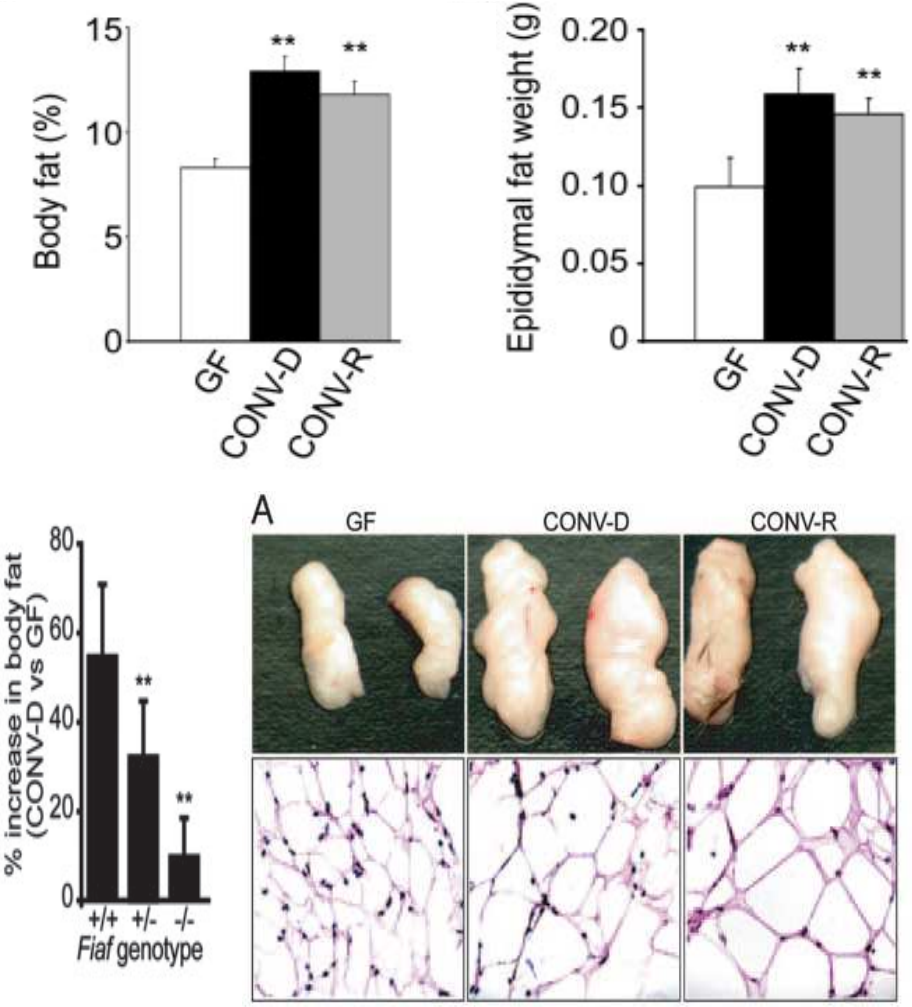
# Gut microbiota ed obesità

**Gordon J. et al. nel 2005 dimostrano che in topi obesi, deficienti nella produzione di leptina (ob-ob), il gut microbiota contiene il 50% in meno di Bacteroidetes and il 50% in più di Firmicutes rispetto ai topi convenzionali.**

# GUT MICROBIOTA e REGOLAZIONE ACCUMULO ENERGIA

- **Topi wild type (WT) mostrano un aumento del 42% del grasso corporeo totale ed un aumento del 47% del grasso gonadico rispetto a topi germ-free (GF);**

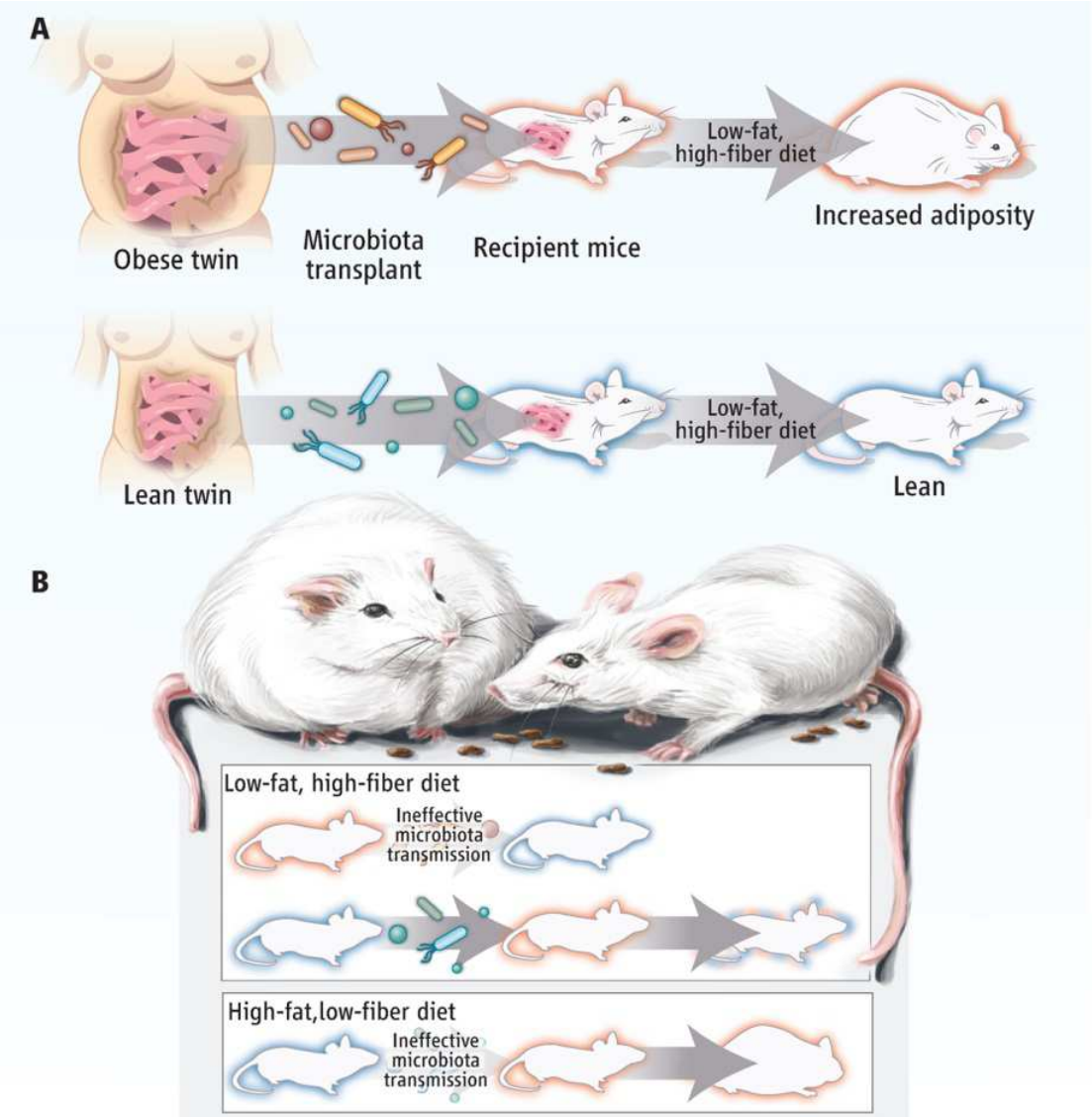
- **Colonizzazione di topi GF con il microbiota proveniente da topi WT si traduce in un aumento del 60% del grasso corporeo totale, e si associa ad un aumentata resistenza all'insulina;**






From: Backhed *et al.* PNAS, 2004

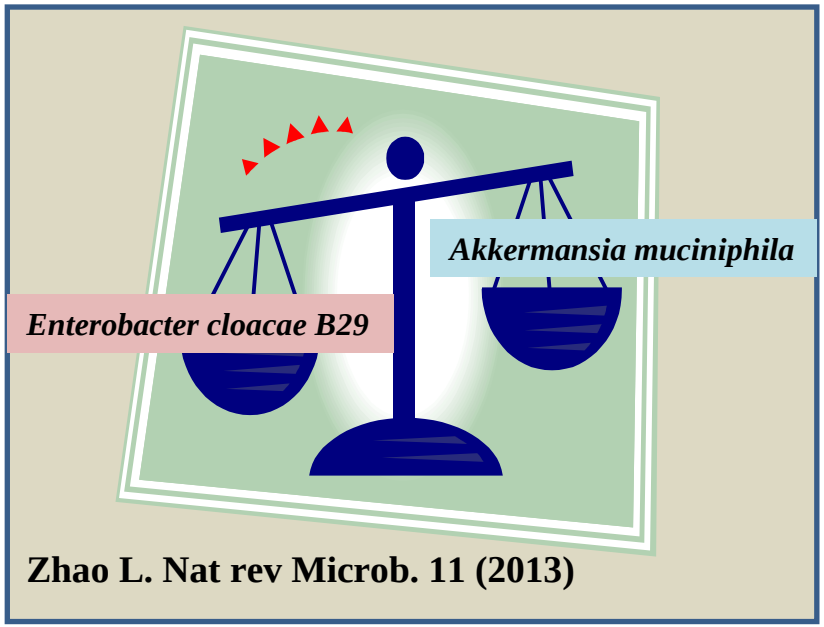
# Il ruolo del gut microbiota nell'obesità



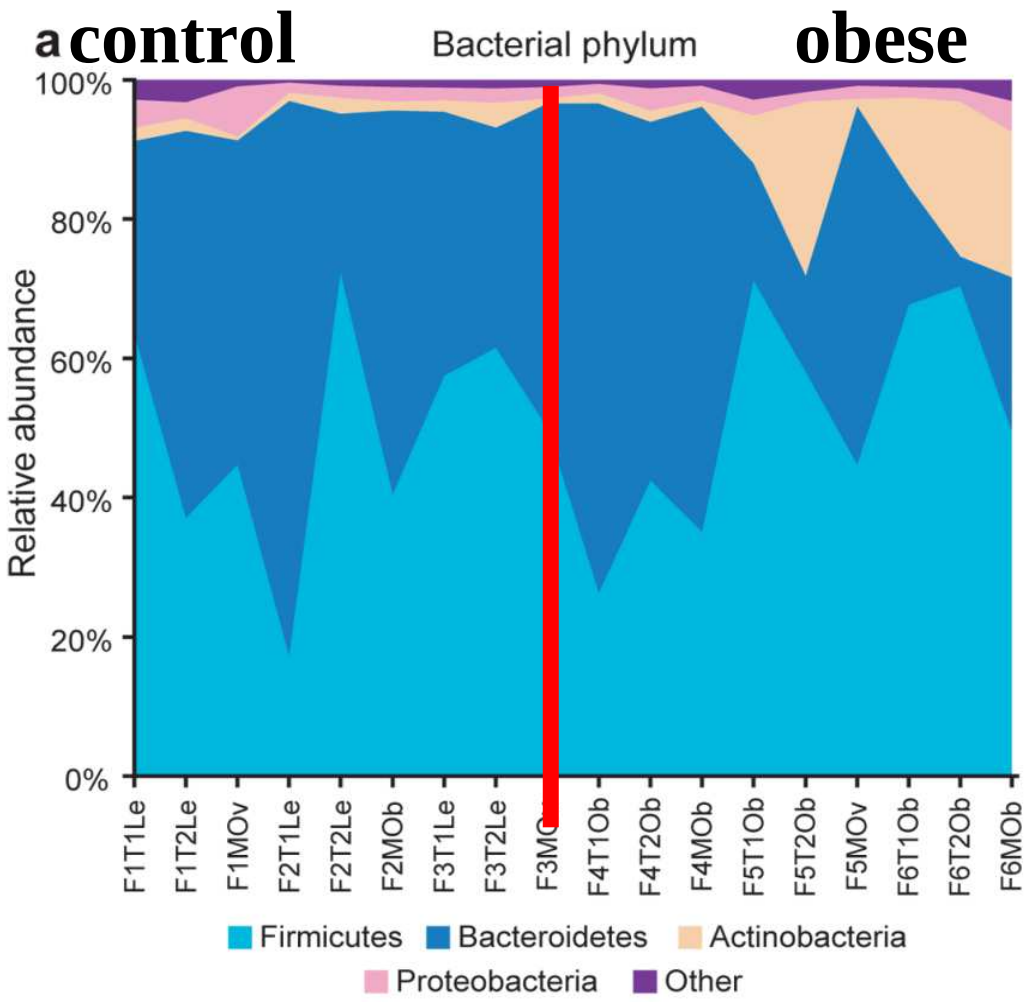
# IL CORE GUT MICROBIOMA negli OBESI

L'obesità è associata ad una ridotta diversità nella composizione batterica, cambiamenti a livello di phylum, ed un alterata composizione di geni batterici e pathways metabolici

***BACTEROIDETES/ FIRMICUTES: indice di adiposità***

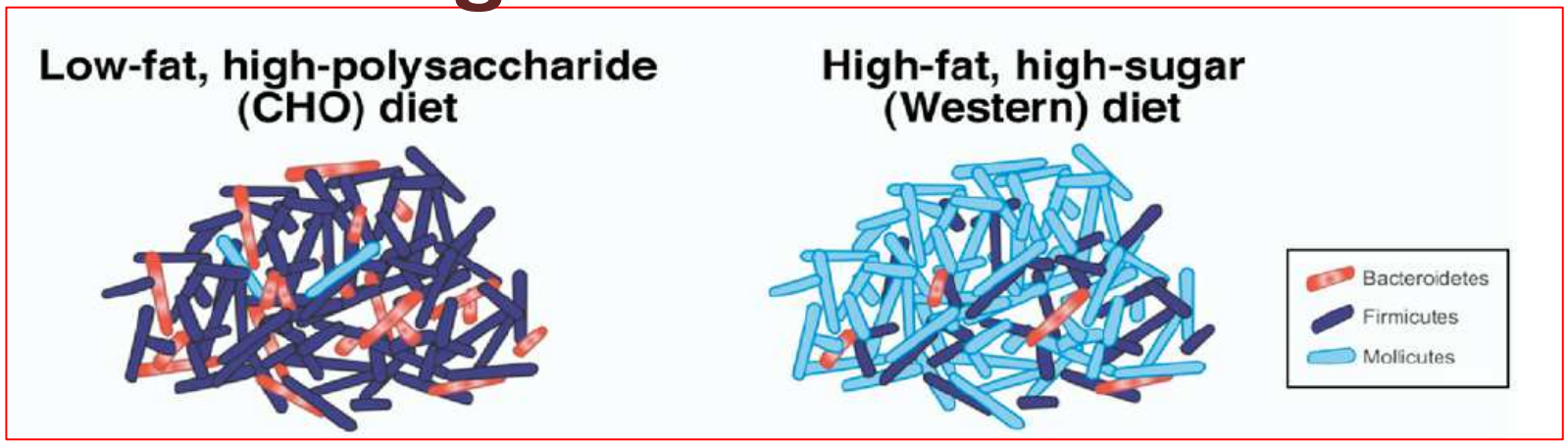



Zhao L. Nat rev Microb. 11 (2013)




Turnbaugh – Nature 2009

# Il ruolo del gut microbiota nell'obesità




***Cambiamenti nell'ecologia microbica nell'intestino***

- Riduzione dei Bacteroidetes ed aumento proporzionale dei Firmicutes
- Crollo drammatico della diversità complessiva
- Aumento di una singola classe di Firmicutes: i *Mollicutes (Erysipelotrichia)*

***Alterazione del potenziale metabolico***

- Arricchimento del sistema delle fosfotransferasi
- Arricchimento dei geni codificanti le beta-fruttosidasi

***Conseguenze***

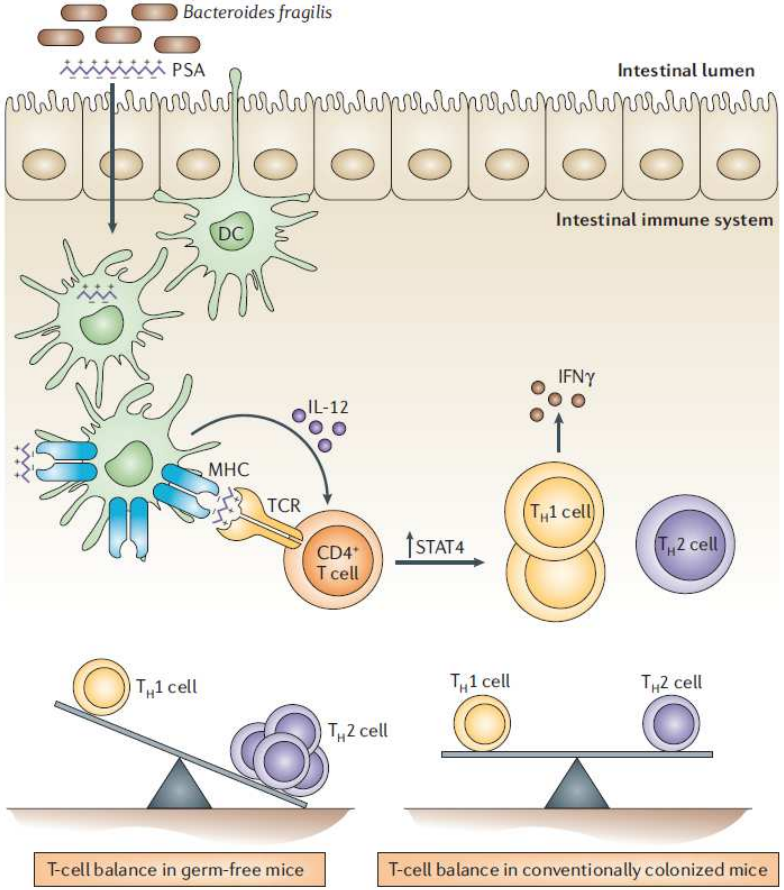
- Aumentata capacità di importare carboidrati tipici della dieta Occidentale
- Aumentata capacità di metabolizzare gli zuccheri importati



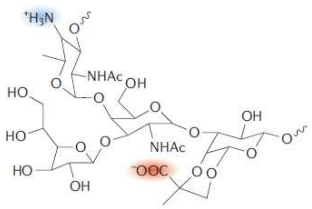
From: Tilg *et al.*, Gastroenterology, 2009

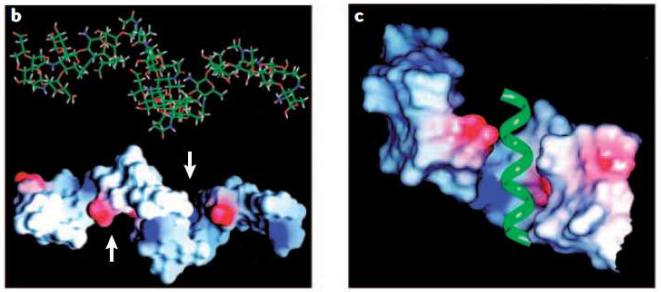
# Principali funzioni del microbiota intestinale

- Digestione dei carboidrati complessi.
- Produzione di vitamine e acidi grassi;
- **Maturazione del sistema immunitario intestinale;**
- **Regolazione della mobilità intestinale;**
- Barriera contro la proliferazione dei patogeni;

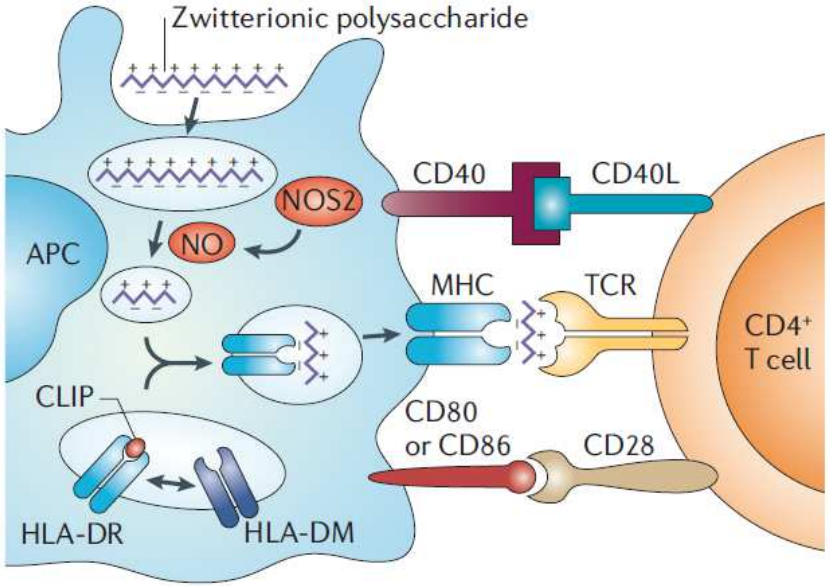


# Ruolo del PSA di *B. fragilis* (capsula) nel corretto sviluppo del sistema immunitario dei mammiferi

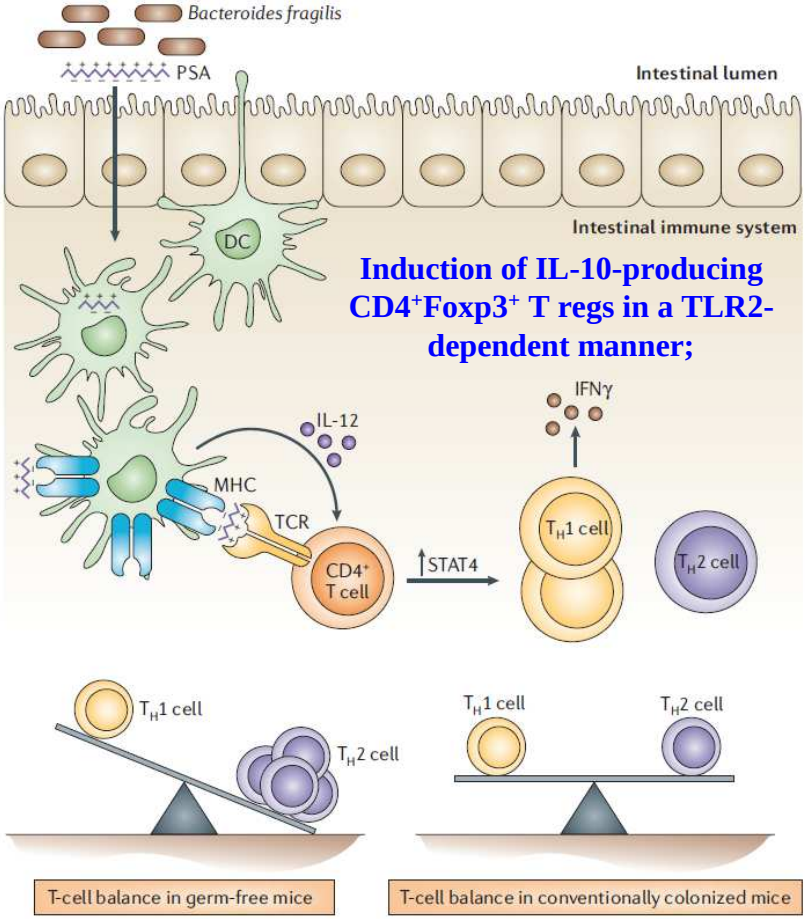





**PSA di *B. fragilis* è composta da diverse centinaia di unità ripetuto di tetrasaccaride. PSA nativo ha un PM di ≅ 130 kDa.**




**La risposta anti-infiammatoria mediata da PSA (abbassa la TH17) è essenziale per promuovere la colonizzazione da parte della flora commensale**




*Mazmanian SK and Kasper DL (2006) Nat Rev Immunol 6, 849 – 858.*

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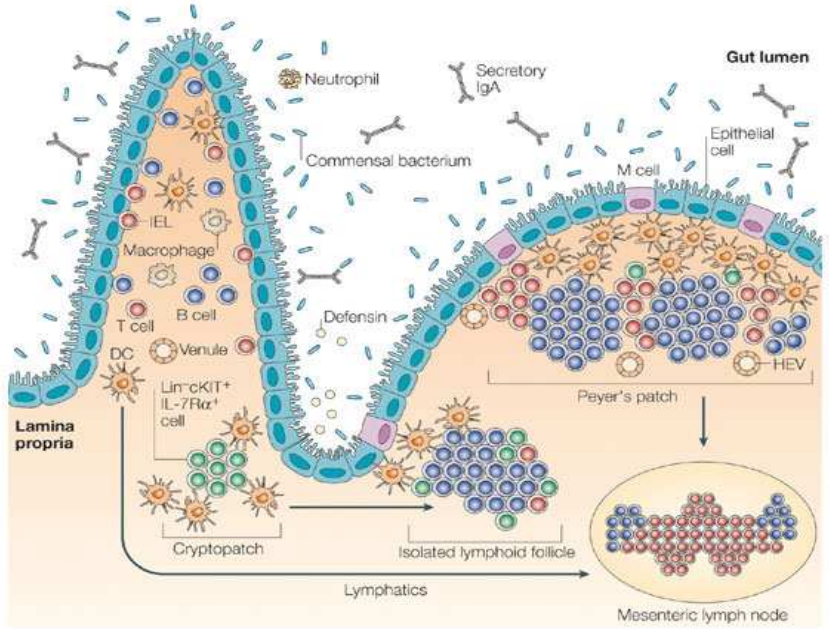
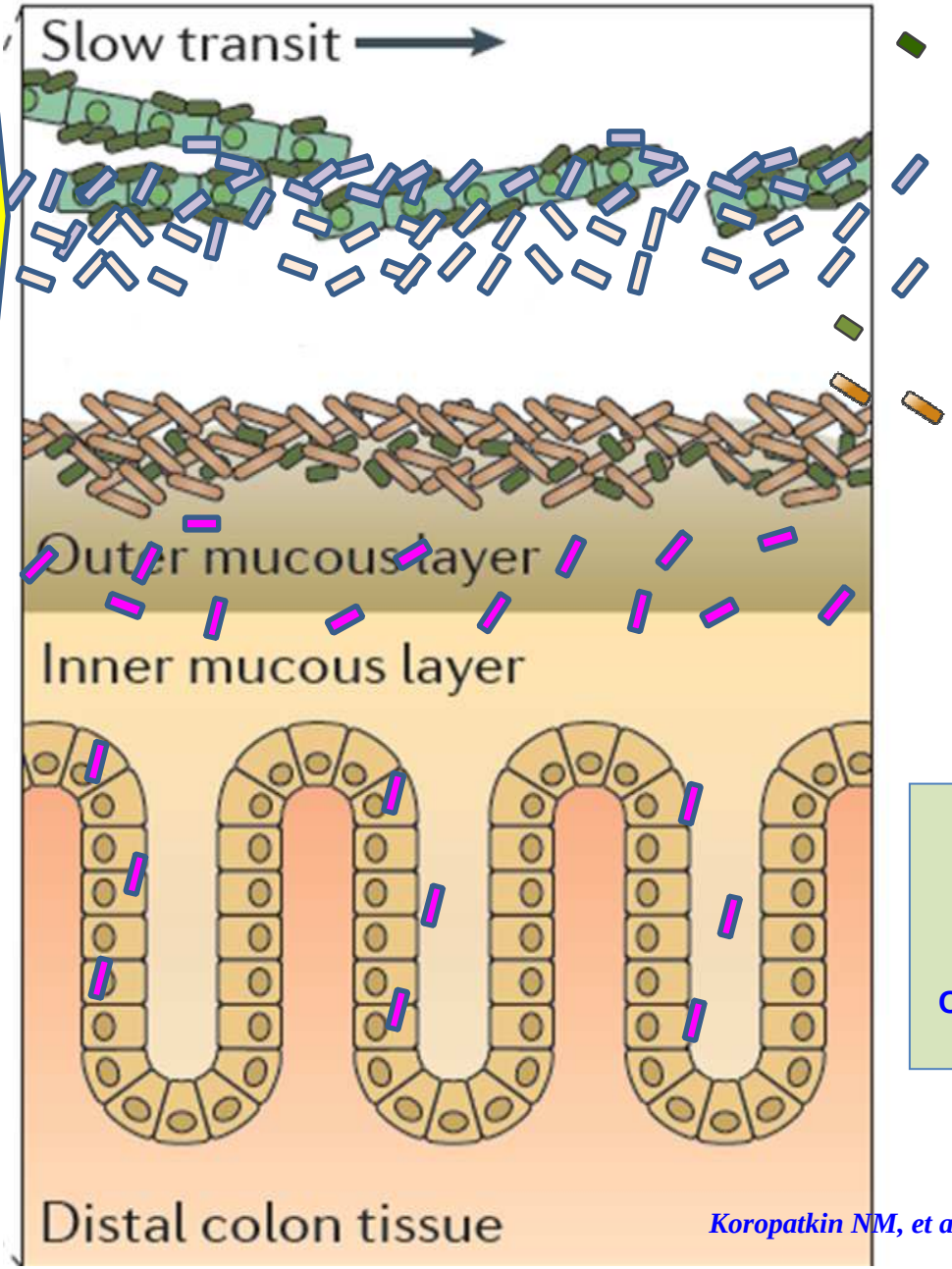
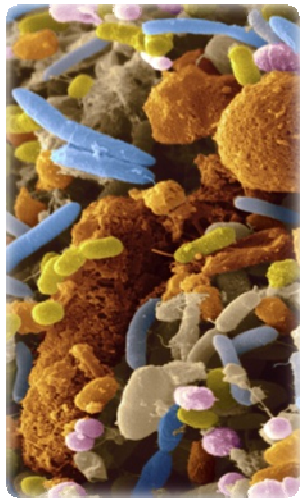



Nature Reviews | Immunology

# Fattori coinvolti nell'equilibrio dinamico del microbiota intestinale

**DIETA**
**ANTIBIOTICI**
**PREBIOTICI**
**INFIAMMAZIONE**
**PATOGENI**



- Altri batteri;
- Fagi;
- Eucarioti;
- Virus;

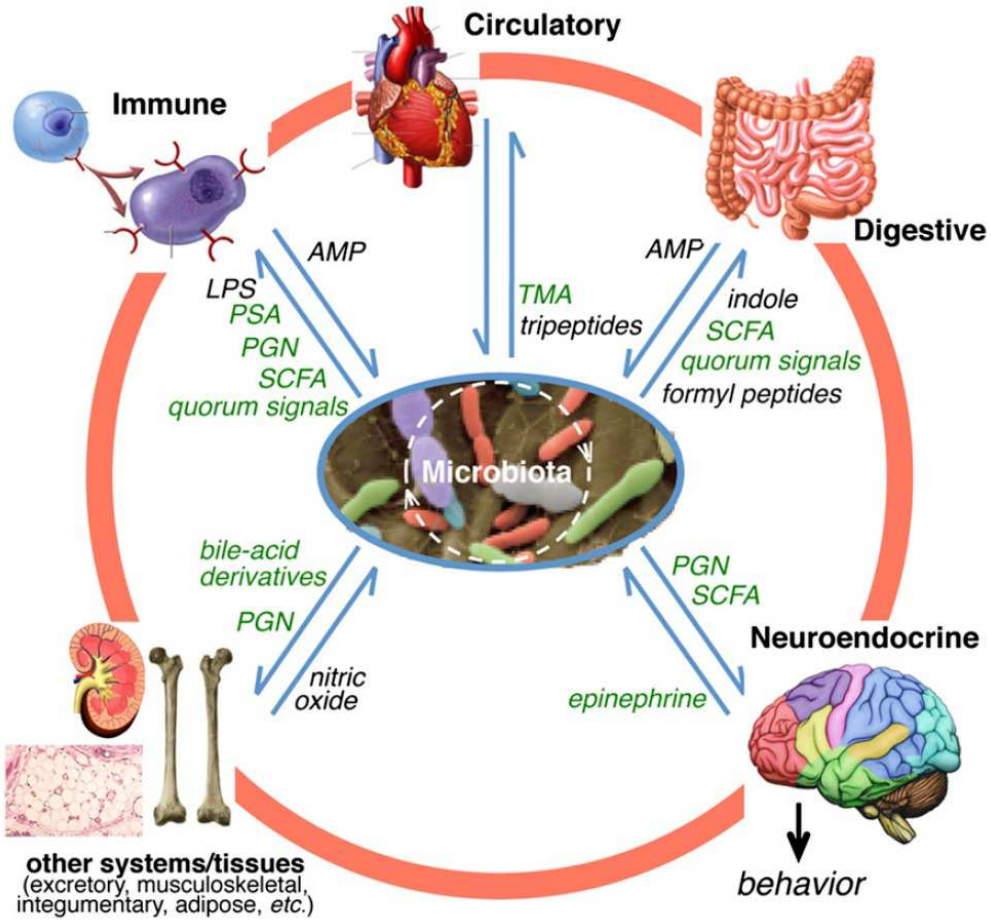
**Variabilità genetica dei batteri (inter ed intra-specie);**

**Competizione/mutualismo**

*Adapted from*
*Koropatkin NM, et al (2012) Nat Rev Microb. 10:323-335*

# Microbiota, organo che interagisce con altri organi

**OMEOSTASI** **MALATTIA**




McFall-Ngai M et al. PNAS 2013;110:3229-3236

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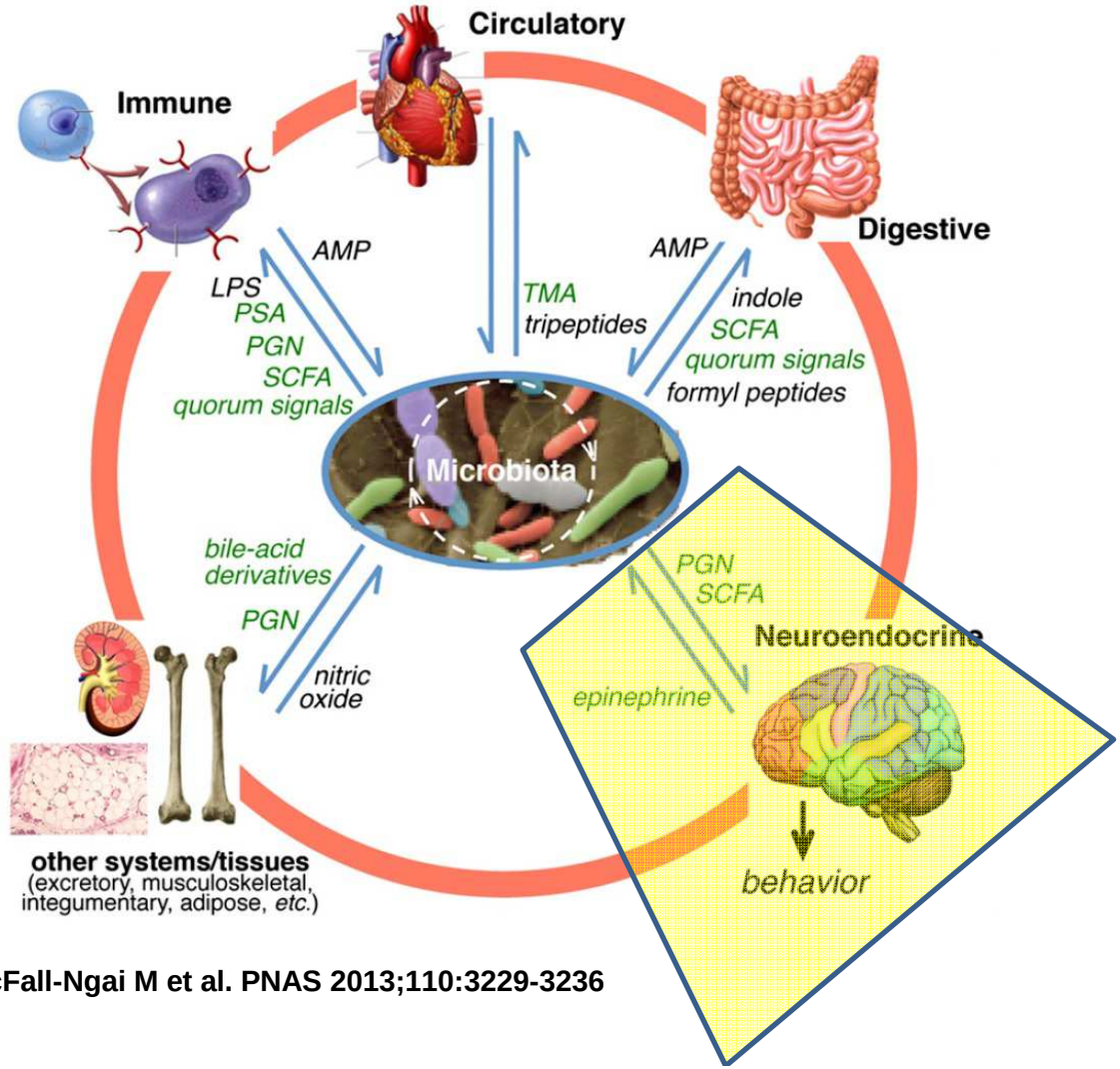
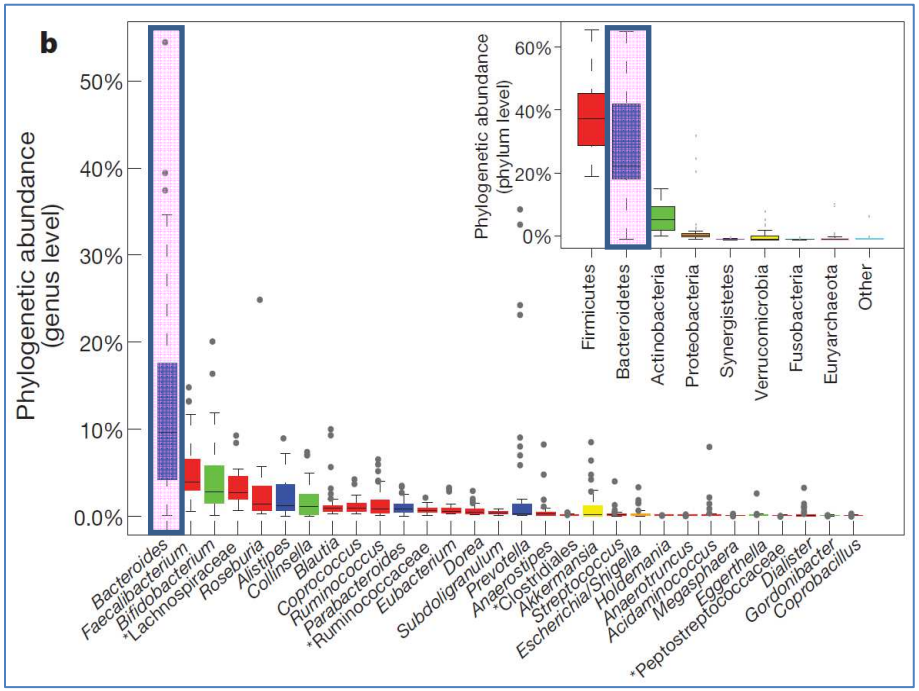



McFall-Ngai M et al. PNAS 2013;110:3229-3236

# Il genere Bacteroides è il più abbondante ma anche il più variabile tra i vari campioni.




- **Singolo phylum dominante (Bacteroidetes) e spesso anche genere (Bacteroides);**
- **Il genere Bacteroides è dominante nelle feci;**
- ***B. thetaiotaomicron* 46% prevalenza;**
- ***B. fragilis* > 0,1% nel 16% dei campioni (>1% nel 3%);**
- **Coinvolto attraverso la capsula nell'immuno-omeostasi;**
- **Degradazione dei polisaccaridi dell'ospite e della dieta;**

**Arumugam M. et al & MetaHIT consortium (2011) Enterotypes of the human gut microbiome. Nature 473, 174-180**

Cell

# Microbiota Modulate Behavioral and Physiological Abnormalities Associated with Neurodevelopmental Disorders

Elaine Y. Hsiao,[1,2,*] Sara W. McBride,[1] Sophia Hsien,[1] Gil Sharon,[1] Embriette R. Hyde,[3] Tyler McCue,[3] Julian A. Codelli,[2] Janet Chow,[1] Sarah E. Reisman,[2] Joseph F. Petrosino,[3] Paul H. Patterson,[1,4,*] and Sarkis K. Mazmanian[1,4,*]
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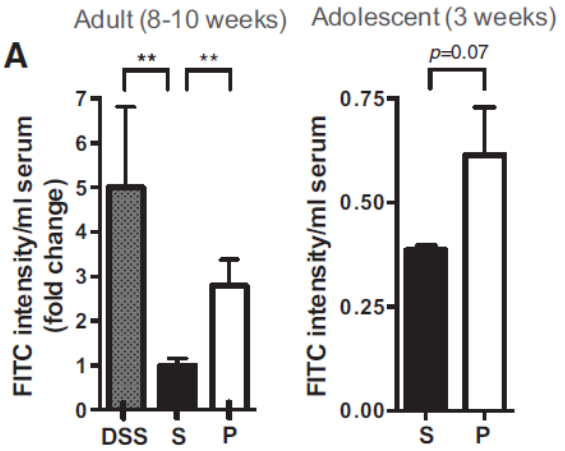
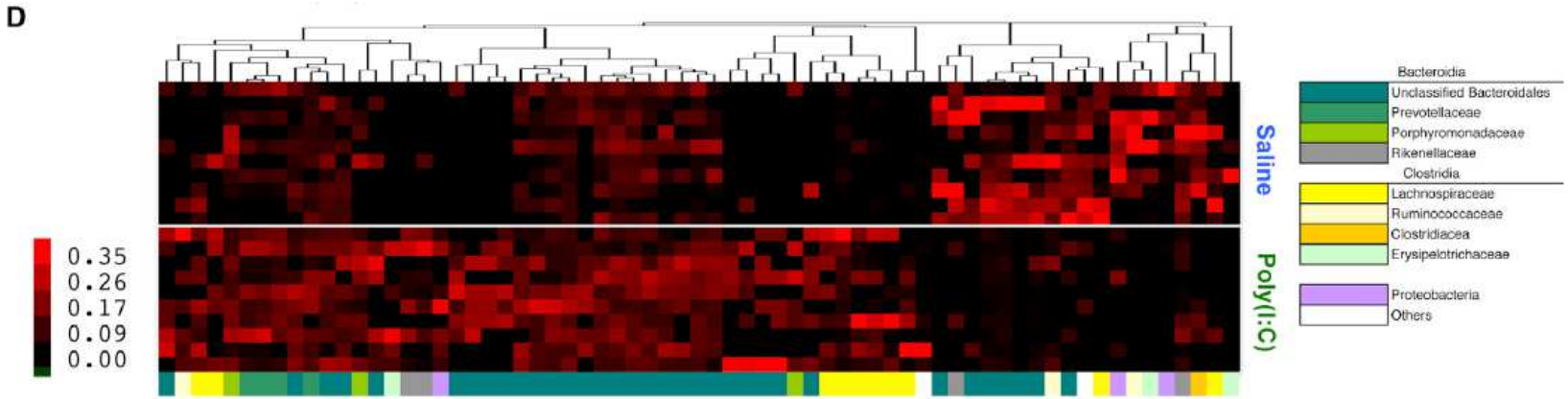
[3]Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX 77030, USA
[4]These authors contributed equally to this work
*Correspondence: ehsiao@caltech.edu (E.Y.H.), php@caltech.edu (P.H.P.), sarkis@caltech.edu (S.K.M.)
http://dx.doi.org/10.1016/j.cell.2013.11.024

## Test di permeabilità intestinale




- **Il modello di autismo viene indotto nei topi mediante la Maternal Immune Activation (MIA), ottenuta attraverso il trattamento delle madri con poly (I:C), che mima un'infezione virale. La prole delle madri MIA mostra i sintomi ed i segni neuropatologici classicamente associati con l'autismo;**

- **Topi con ASD (Autism Specrum Disorders) mostrano ridotta integrità intestinale, abnorme produzione di citochine ed alterato microbiota intestinale, che coinvolge Clostridia e Bacteroides;**



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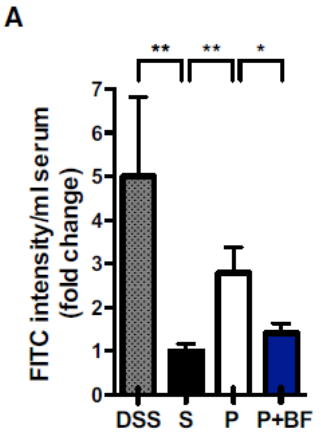
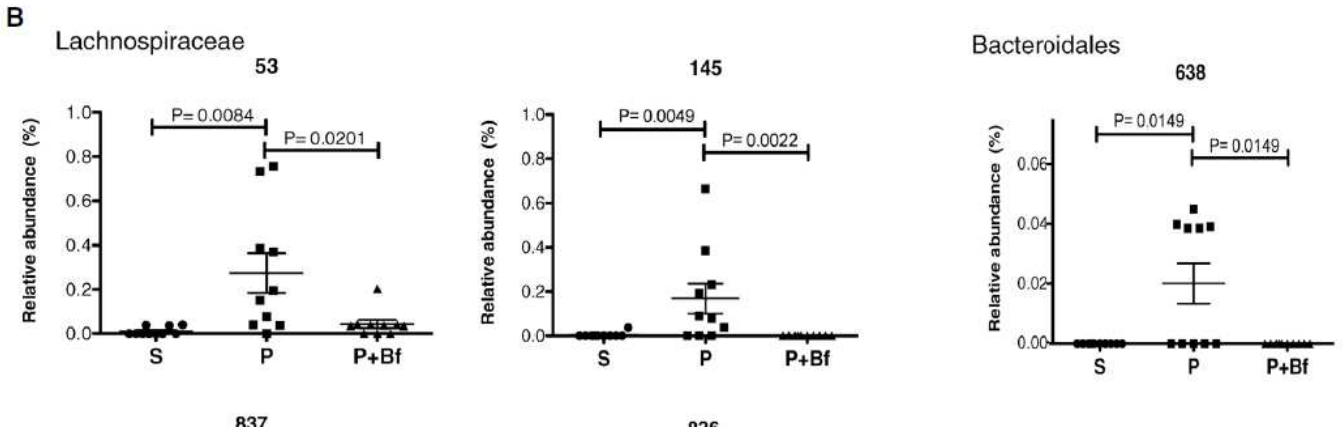
**Saggio di permeabilità intestinale;**




- **Il trattamento durante lo svezzamento con *B. fragilis* della prole delle madri MIA migliora l'integrità della barriera intestinale e corregge le alterazioni nella composizione del microbiota e produzione di citochine;**

- **Tali cambiamenti si verificano in assenza di una persistente e misurabile colonizzazione da parte di *B. fragilis*;**



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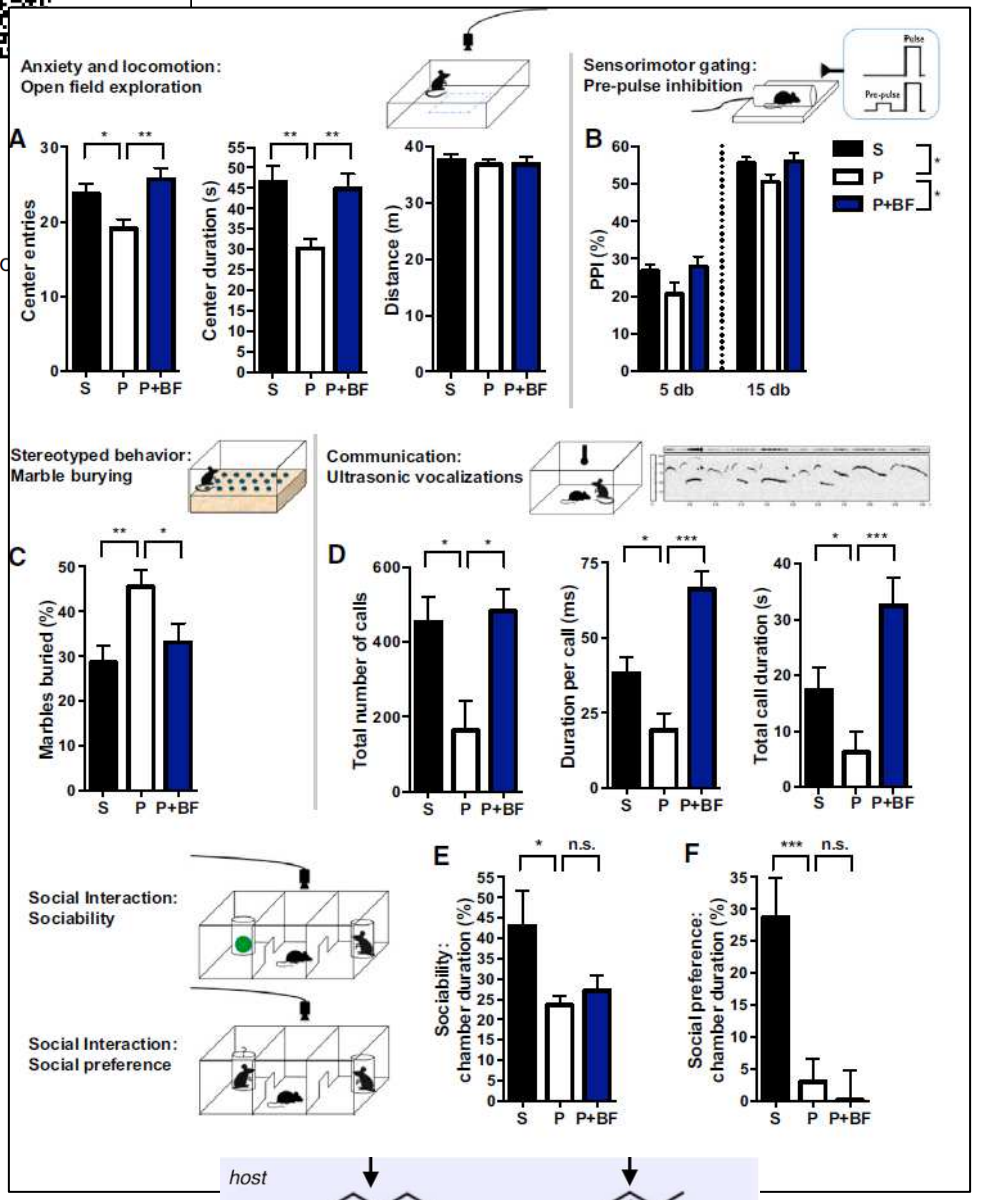
- **La prole delle madri trattati con MIA presentano una serie di anormalità classicamente associate all'autismo;**
- **Tali anormalità vengono corrette dal trattamento con *B. fragilis*;**
- **Risultati simili sono stati ottenuti quando i topi sono stati trattati con *B. thetaiotaomicron* ma non con *Enterococcus faecalis*;**
- **La prole delle madri MIA mostrava un aumento di 46 volte di alcuni metaboliti nel siero (4EPS simile al p-cresol, che si osserva nei bambini con autismo) e tale anormalità veniva corretta a seguito del trattamento con *B. fragilis*;**
- **Il trattamento con 4EPS induce ASD;**



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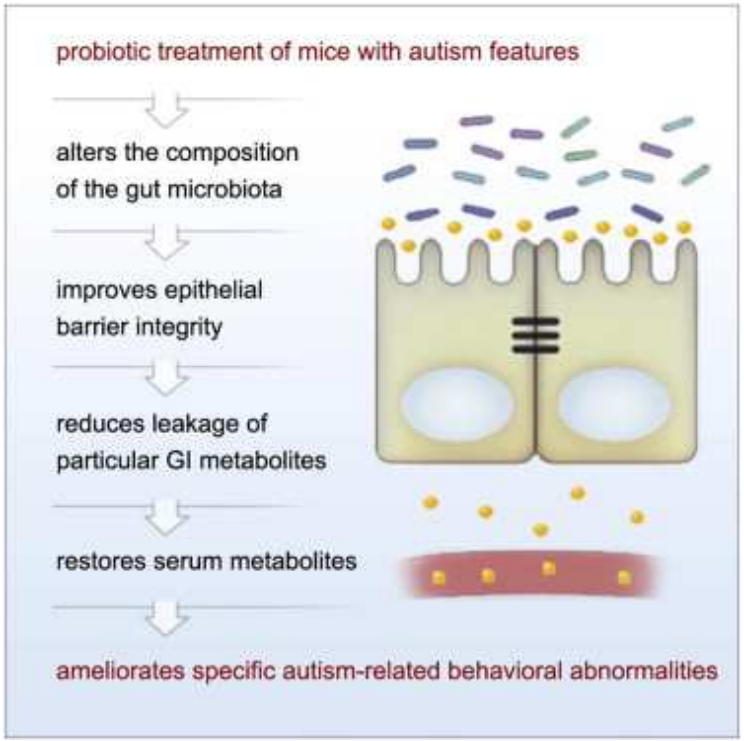


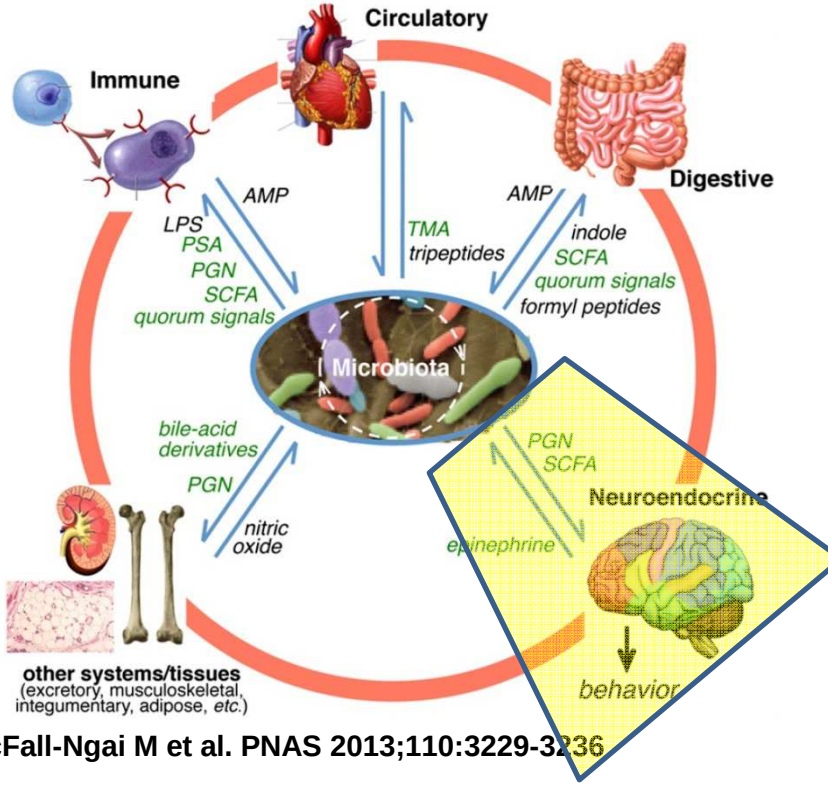


McFall-Ngai M et al. PNAS 2013;110:3229-3236

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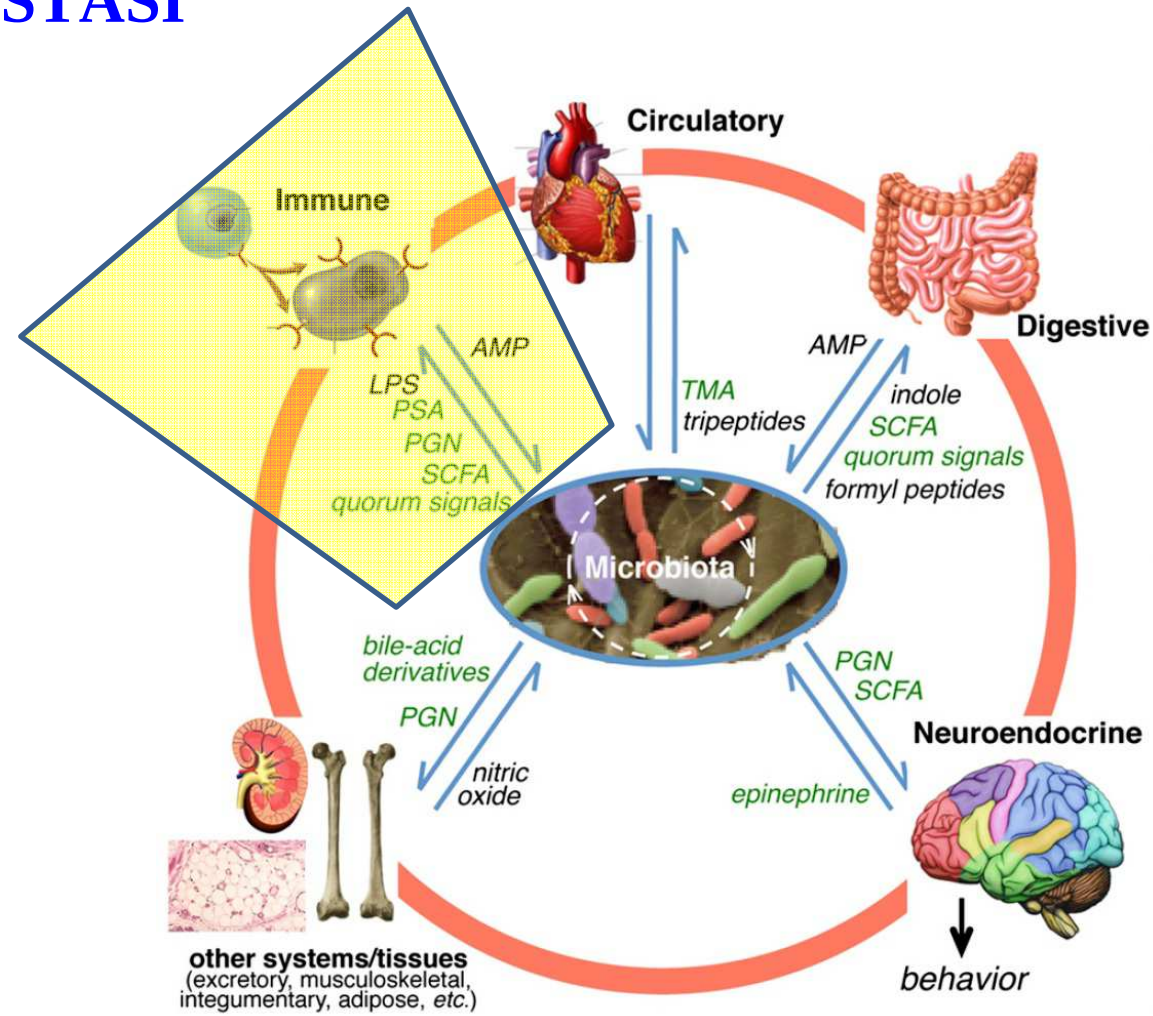


# Modulazione della risposta pro-infiammatoria da parte del microbiota

| | |
|---|---|
| **Luminal PAMPs*** | ***Specific PRRs*** |
| ✓ LPS (Gram -) | TLR-4 |
| ✓ UPEC/Profilin | TLR-11 |
| ✓ Flagellin | TLR-5 |
| ✓ Peptidoglican/lipopeptide | TLR-1 e TLR-2 |
| ✓ Bacterial lipopeptide | TLR-2 e TLR-6 |
| ✓ ds RNA | TLR-3 |
| ✓ Fibronectina (many bacteria) | $\alpha 5 \beta 1$ integrin |
| ✓ Lipothecoic acid (Gram +) | TLR-2 |
| ✓ Lipooligosaccharide | PAF |
| **Endosomial PAMPs** | **Specific PRRs** |
| ✓ ss RNA | TLR-6 e TLR-7 |
| ✓ CpG DNA | TLR-9 |

From: Balfour and Sartor, Gastroenterology 2008, modified

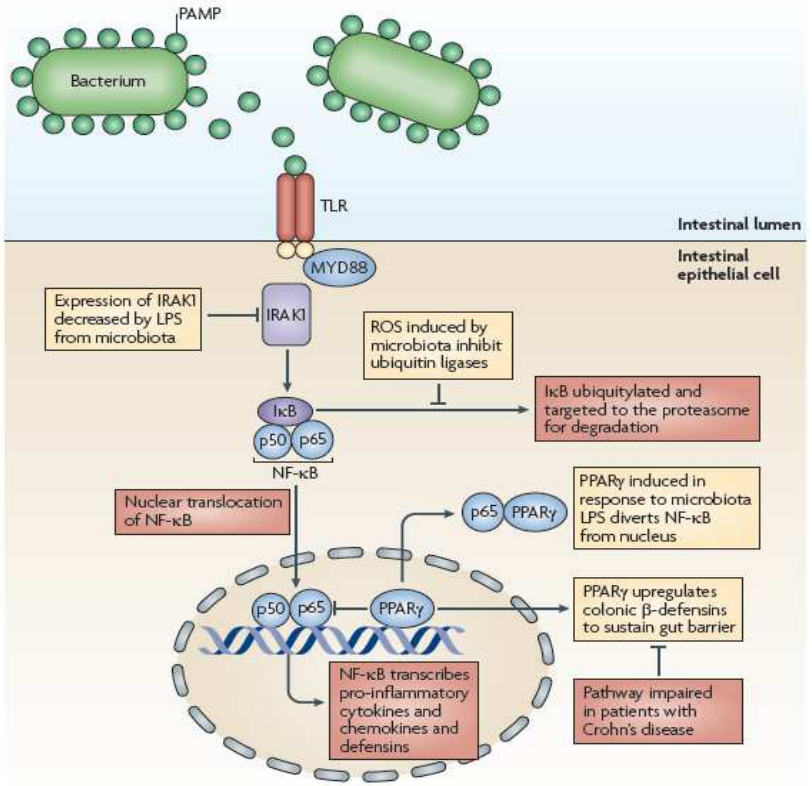
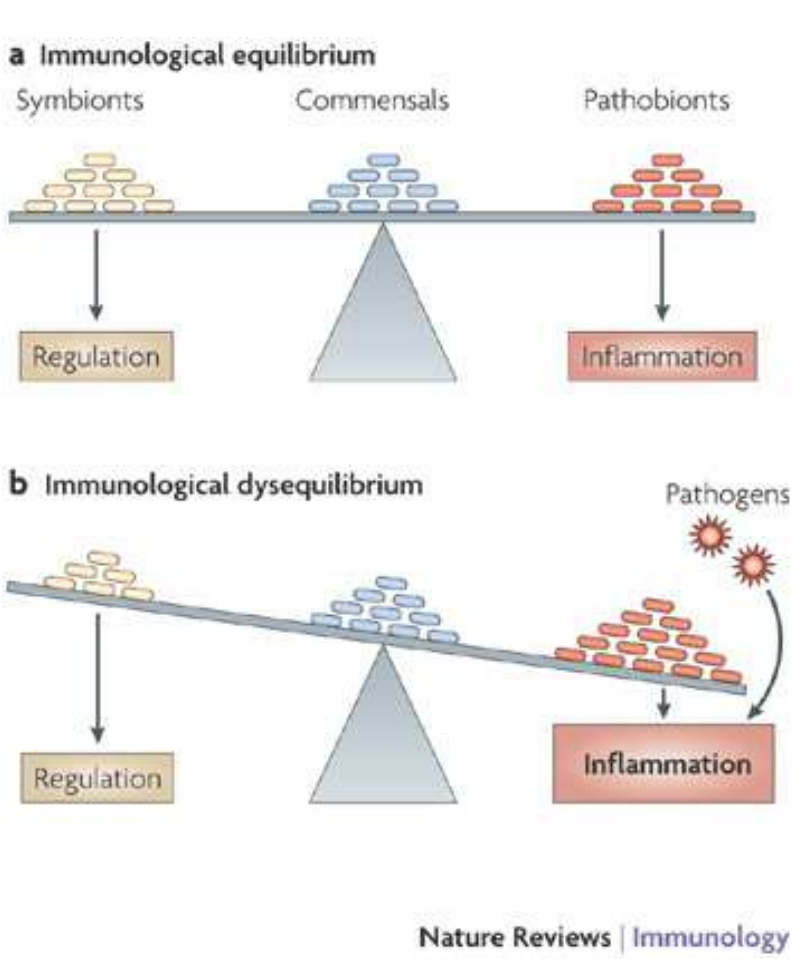



Figure 1 | **Modulation of intestinal epithelial cell pro-inflammatory responses by the microbiota.**

*** PAMPS: Pathogen Associated Molecular Patterns; **PRRs: Pathogen Recognition Receptors;**

# Interazioni ospite-microbiota



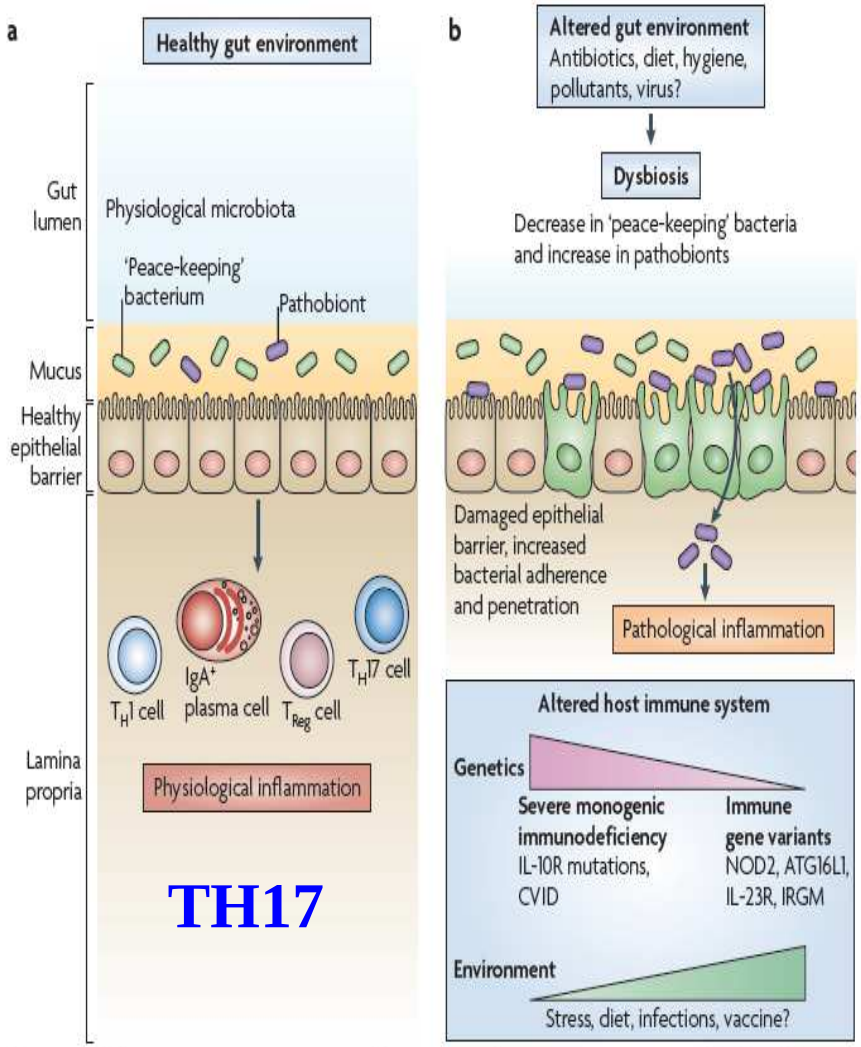



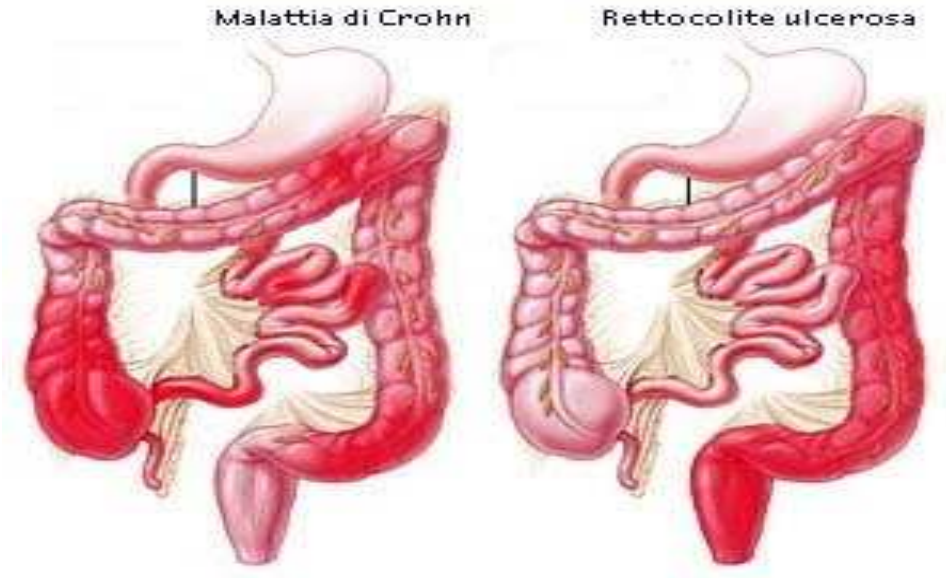

Figure 4 | **Schematic representation of host–microbiota interactions in the healthy and inflamed gut. a** | In healthy hosts, an efficient immune barrier contains the microbiota in the gut

# Microbiota intestinale

**Alterazioni del Microbiota intestinale (disbiosi)**

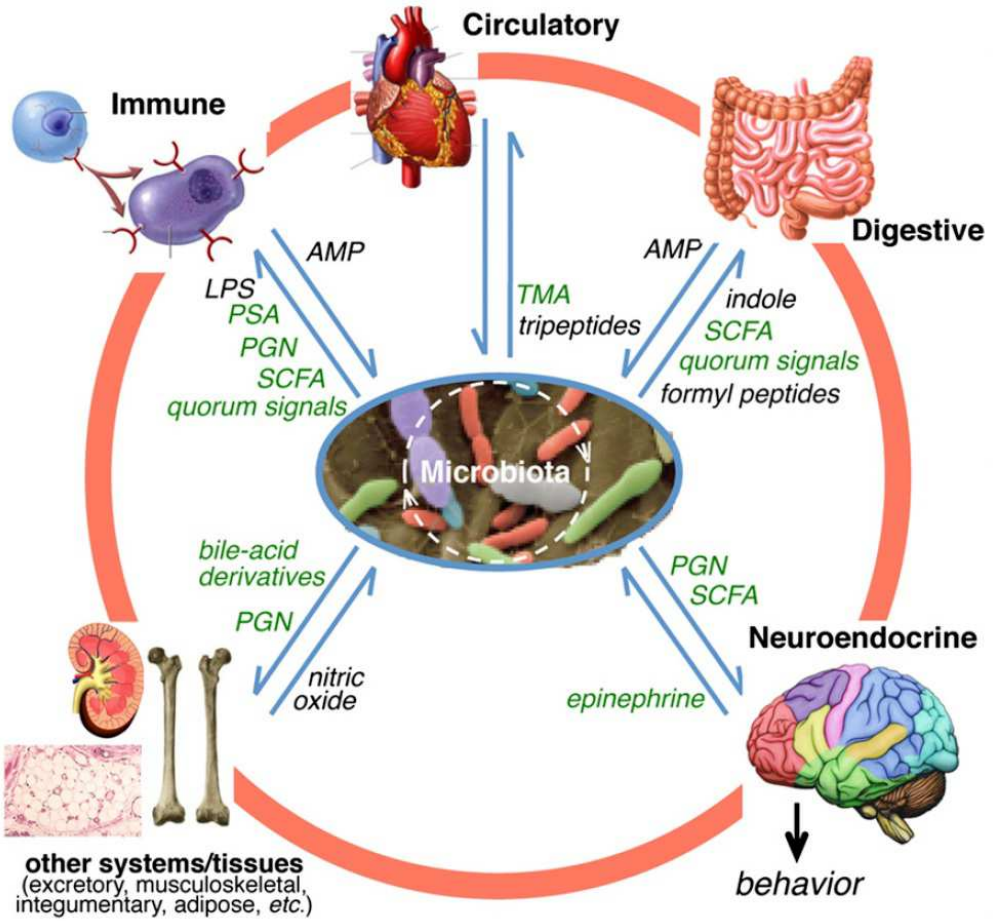
**Alterazioni del sistema immunitario**

- **Obesità;**
- **Malattie autoimmuni;**
- **Mal. Infiammatorie (IBD);**

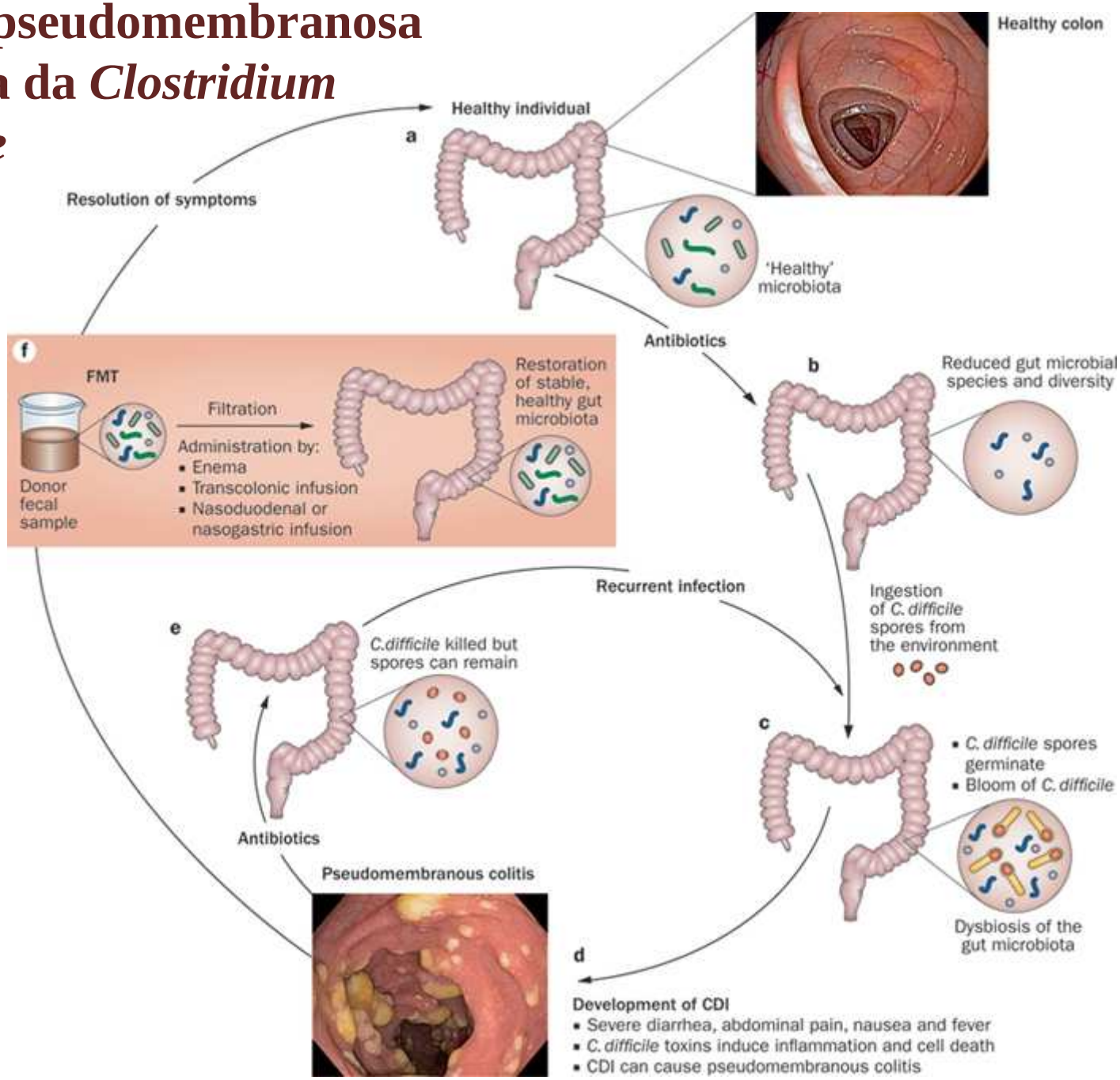


# Microbiota, organo che interagisce con altri organi

**OMEOSTASI** ⇄ **MALATTIA**



# Colite pseudomembranosa causata da *Clostridium difficile*



The NEW ENGLAND JOURNAL of MEDICINE
January 16, 2013

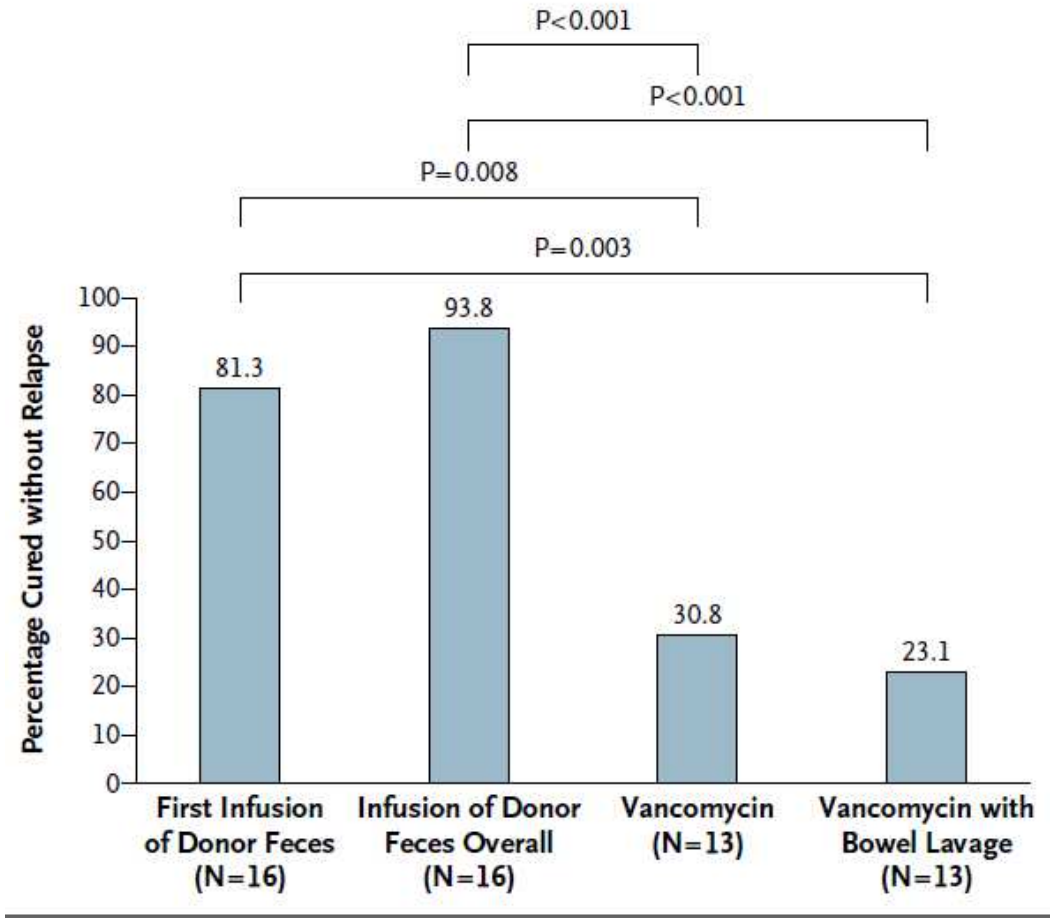
# Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

E. van Nood, A. Vrieze, M. Nieuwdorp, S. Fuentes, E.G. Zoetendal,
W.M. de Vos, C.E. Visser, E.J. Kuijper, J.F.W. M. Bartelsman,
J.G.P. Tijssen, P. Speelman, M.G.W. Dijkgraaf, and J.J. Keller.

From:
the Departments of Internal Medicine (E.N., A.V., M.N., P.S.),
Microbiology (C.E.V.),
Gastroenterology ( J.F.W.M.B., J.J.K.),
and Cardiology ( J.G.P.T.)
and the Clinical Research Unit (M.G.W.D.),
Academic Medical Center, University of Amsterdam, Amsterdam;
the Laboratory of Microbiology, Wageningen University, Wageningen (S.F., E.G.Z., W.M.V.);
the Department of Experimental and Medical Microbiology, Leiden University Medical Center, Leiden (E.J.K.);
and the Department of Gastroenterology, Hagaziekenhuis, The Hague ( J.J.K.)
- all in the Netherlands -

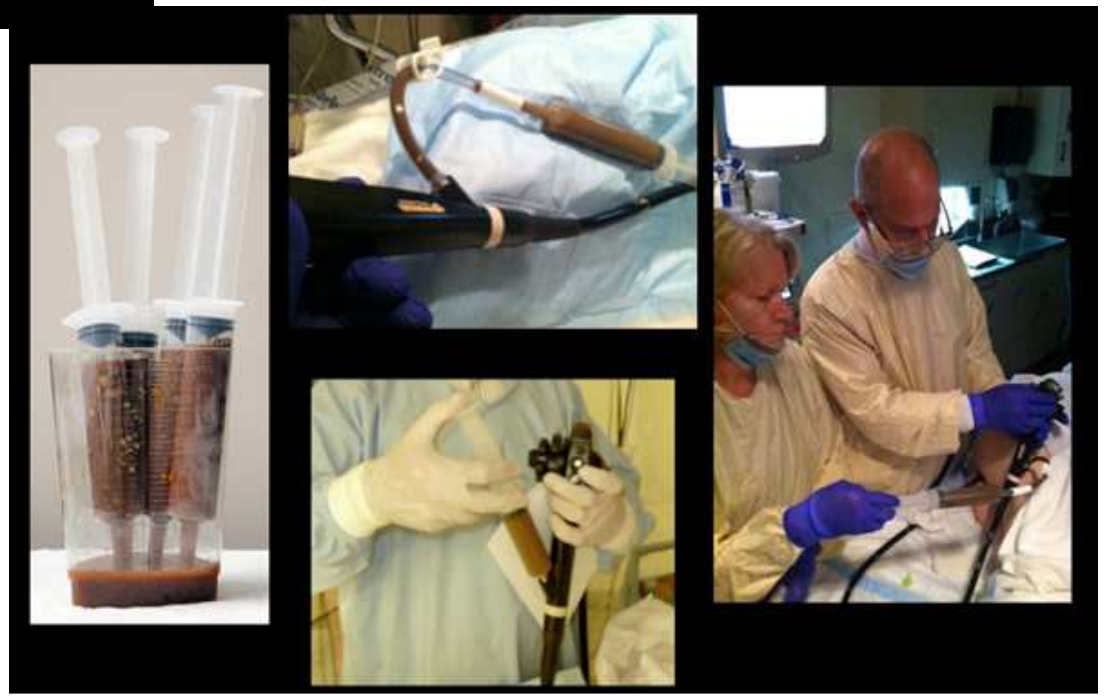
and the Department of Bacteriology and Immunology, Medical Faculty, University of Helsinki, Helsinki (W.M.V.).

# Tassi di cura senza relapse in soggetti con infezione ricorrente da *Clostridium difficile*




**Five weeks after** the initiation of therapy, there was a recurrence of infection in 1 of 16 patients (6%) in the infusion group, 8 of 13 (62%) in the vancomycin-alone group, and 7 of 13 (54%) in the group receiving vancomycin with bowel lavage.

Of 16 patients in the infusion group, 13 (81%) were cured after the first infusion of donor feces. The 3 remaining patients received a second infusion with feces from a different donor; of these patients, 2 were subsequently cured. Overall, donor feces cured 15 of 16 patients (94%).
Resolution of infection occurred in 4 of 13 patients (31%) in the vancomycin-alone group and in 3 of 13 patients (23%) in the group receiving vancomycin with bowel lavage.
The overall cure rate ratio of donor-feces infusion was 3.05 as compared with vancomycin alone (99.9% confidence interval [CI], 1.08 to 290.05) and 4.05 as compared with vancomycin with bowel lavage



UNIVERSITÀ CATTOLICA DEL SACRO CUORE
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*COMITATO ETICO*

Prot.sf 8440/13

UCSC PROTOCOLLO GENERALE
Prot. n. 11107/13
Del 15/05/2013

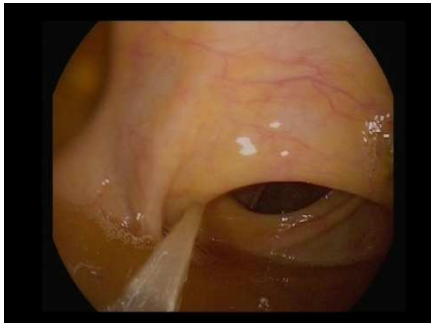
Approvato !

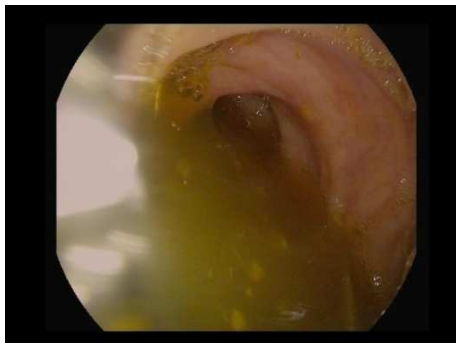
# Il trapianto fecale nel trattamento della ricorrenza dell'infezione da *Clostridium difficile*

# Trapianto fecale nell'infezione ricorrente da *Clostridum difficile*

**2013, prima esperienza in Italia**

| | Gender | Age (yrs) | Number of CD recurrences | CD colture/ toxin before treatment | Donor | Route of infusion | Fecal quantity (gr)/volume of infusion (mL) | Resolution of symptoms/ FMT related side effect | CD toxin after treatment | Follow-up time |
|---|---|---|---|---|---|---|---|---|---|---|
| Case 1 | M | 82 | 5 | Positive/ positive | Daughter | Colonoscopy | 200/400 | Yes /none | Negative | 4 months |
| Case 2 | M | 75 | 2 | Positive/ positive | Son | Colonoscopy | 180/420 | Yes /none | Negative | 4 months |
| Case 3 | F | 24 | 1 | Positive/ positive | Brother | Colonoscopy | 180/500 | Yes /none | Negative | 3 months |
| Case 4 | F | 84 | 3 | Positive/ positive | Son | Colonoscopy | 200(420 | Yes /none | | 1 week |







Cammarota *et al*. Dig. Liv. Dis., 2014

# Storia del trapianto fecale

- La prima descrizione sull'uso delle feci umane come rimedio per alcune malattie la troviamo in un antico testo di medicina cinese; autore Ge Hong, circa 1700 anni fa.

- Successivamente, l'assunzione di feci di cammello fresche e calde veniva raccomandata dai Beduini come rimedio per la dissenteria batterica; l'efficacia del trattamento venne confermata dai soldati tedeschi in Africa durante la II guerra mondiale;

- Nella medicina moderna, l'infusione di feci da donatori sani venne adottata per la prima volta nel 1958, ad opera del Dr. Eiseman che descrive l'eroica risposta in pazienti con diarrea associata al antibiotico-terapia e successivamente trattati con enema contenente feci di donatori.

# Microbiota: questo sconosciuto

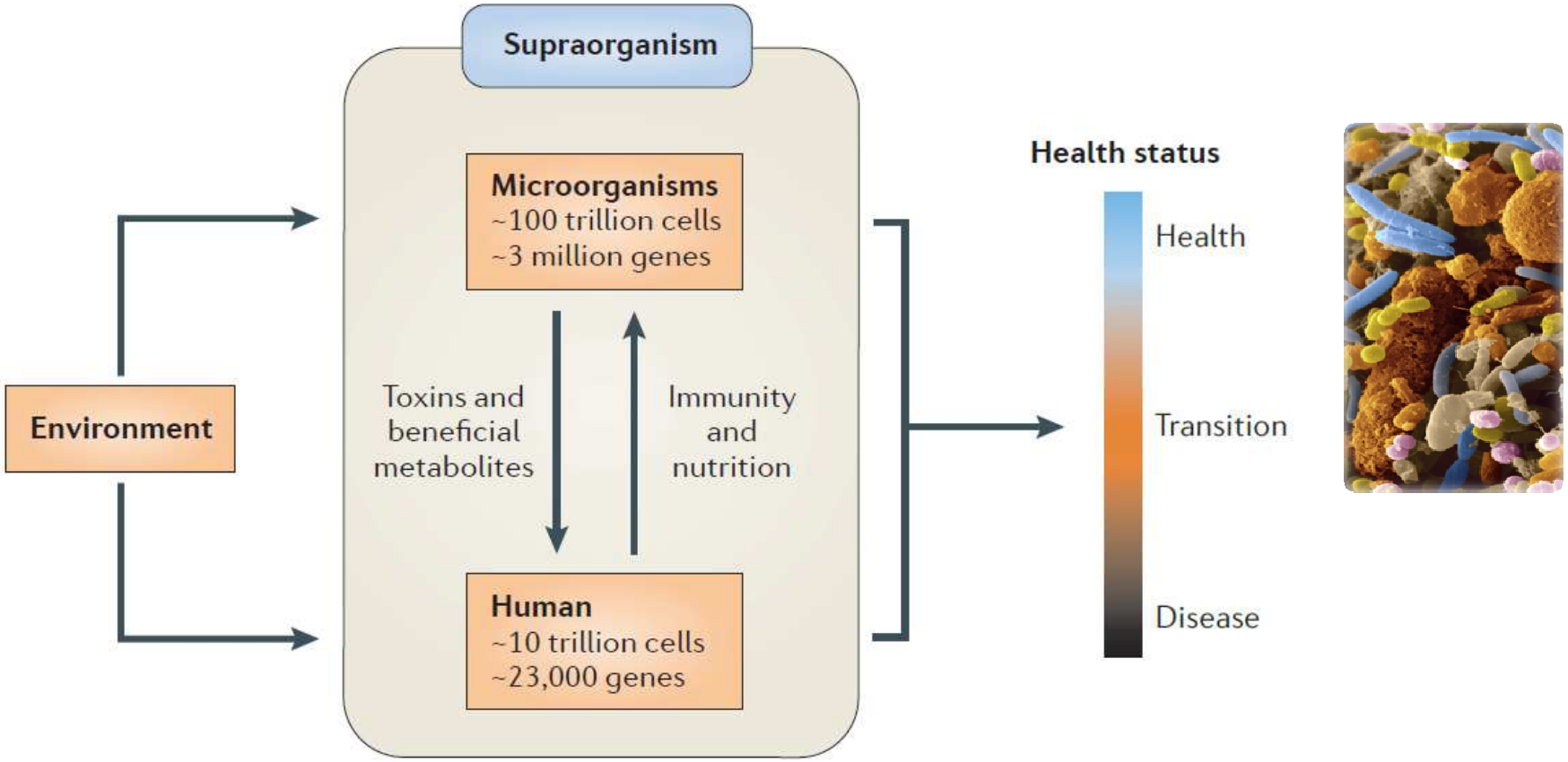
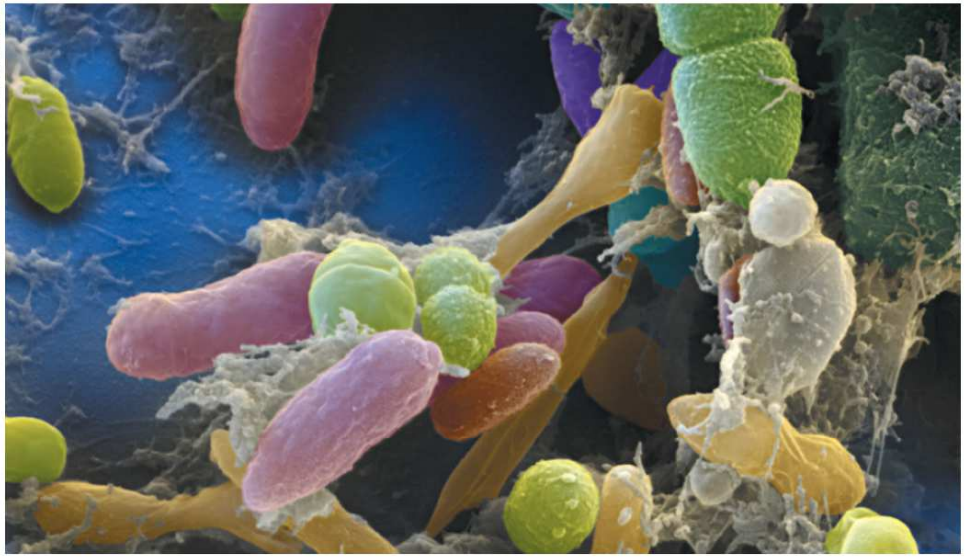



Figure 1 | **Human health is influenced by interactions among the gut microbiota, the host and the environment.** Humans are supraorganisms consisting of both human cells and microbial cells,

**Zhao L. (2013) Nat. Rev. Microbiol. 11: 639-647**

EYE OF SCIENCE/SPL



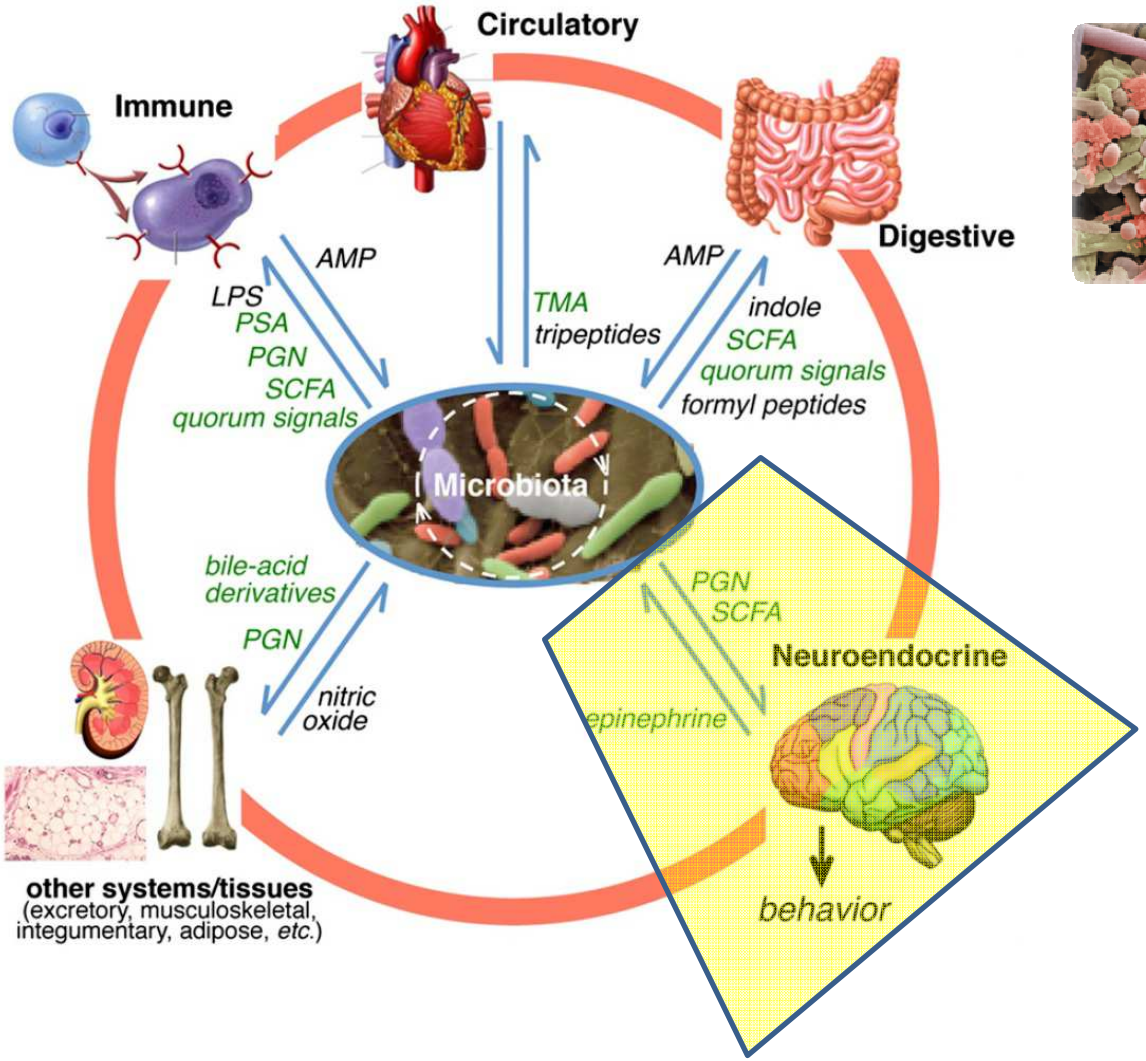
A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

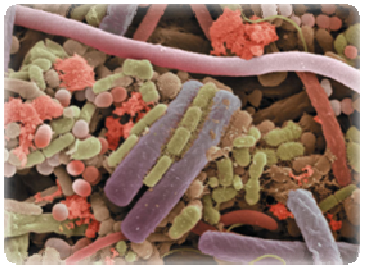
# Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says **William P. Hanage**.

*Grazie per l'attenzione!!!*

# Microbiota, organo che interagisce con altri organi



McFall-Ngai M et al. PNAS 2013;110:3229-3236