

VI Congresso Regionale F.I.M.P Marche



Novità nella ricerca sul microbiota intestinale umano e la modulazione con i probiotici

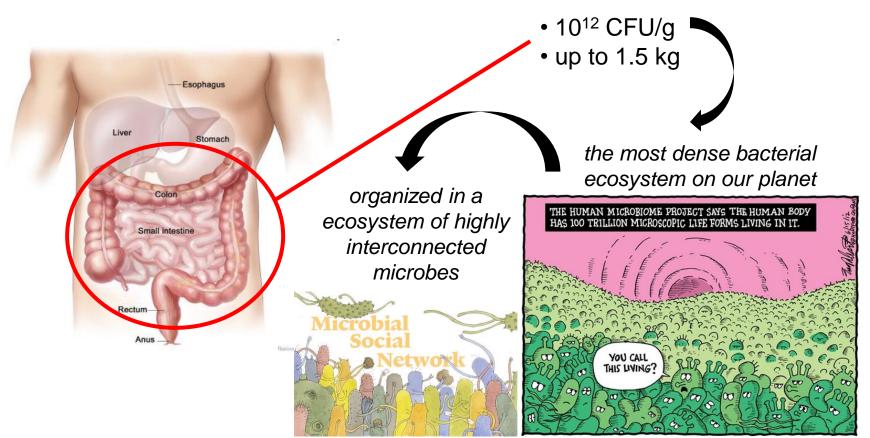
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Fano, 24 Ottobre 2015



10¹³–10¹⁴ habits our body and the great majority of these microorganisms is hidden in the gastrointestinal tract





GIT MICROBIOTA: THE MOST COMPLEX ECOSYSTEM



Human organism contains 10^{13} cells = 10.000 billion and harbors a microbial population of 10^{14} cells = 100.000 billion 1 human cell
10 microbial cells 1 human gene 🔿 150 microbial genes we are 90% bacteria $(10^{13}-10^{14})$ **METAORGANISM** result of millennia of co-evolution

with an incredible number of symbiont microbial cells living in our body



HOW DO WE KNOW THIS?

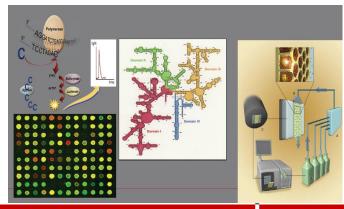
Culture-based methods allow to recover 20-30% of total microscopic counts





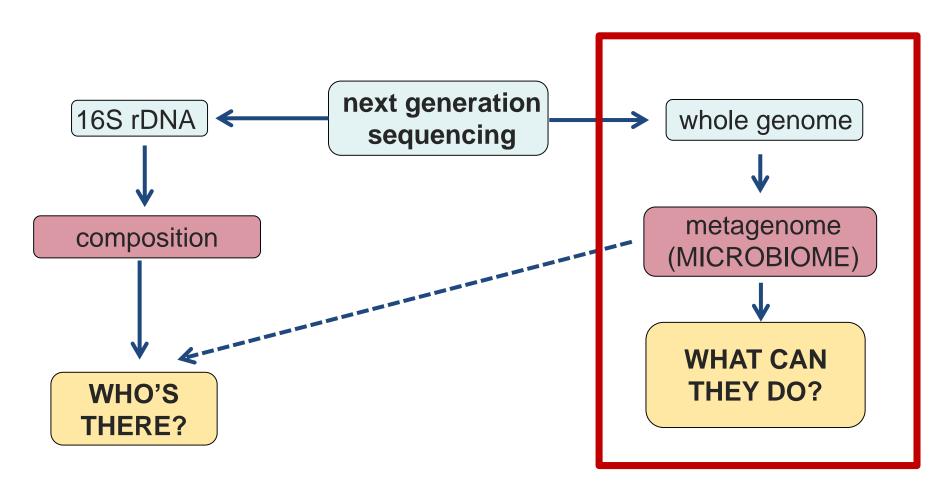
MOSTLY UNCULTURED!

- Culture-independent molecular survey:
- DNA extraction from gut samples
- 16S based molecular techniques



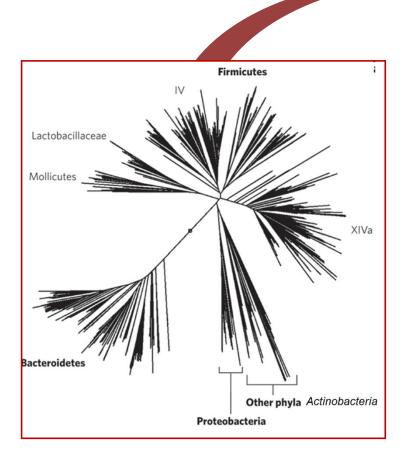


MICROBIOTA MOLECULAR ASSESSMENT





PHYLOGENETIC DIVERSITY



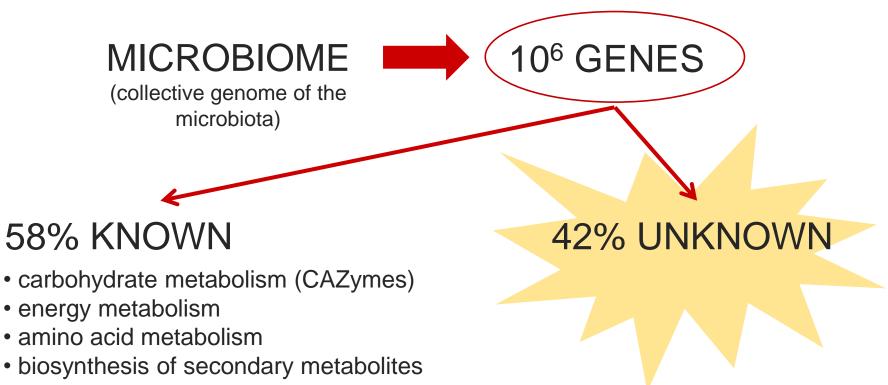
> 1000 species

6 (out of 100) bacterial phyla

- Firmicutes, Bacteroidetes : 90%
- Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia : 10%



FUNCTIONAL DIVERSITY

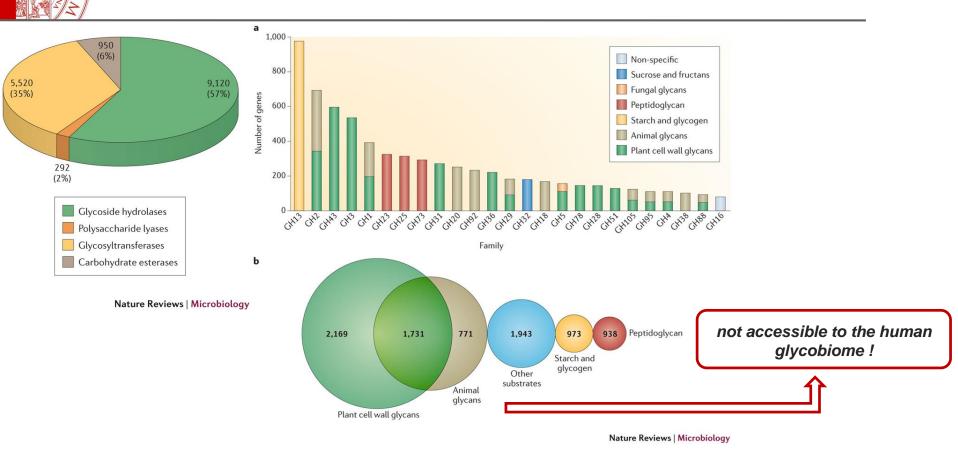


metabolism of cofactors and vitamins

Schloissing et al, Nature 2013

SUBSTRATES OF THE GM CAZymes ARSENAL

thousands of enzymes while we possess only 17



The GM possesses a broad glycobiome complexity, complementing the limited diversity of the human glycobiome and enhancing the superorganism capacity to metabolize complex polysaccharides

El Kaoutari et al., Nat Rev Microbiol. 2013



IMPACT OF MICROBIOTA ON HOST HEALTH AND DISEASE

our bacterial counterpart provides essential features we have not evolved

- <u>enhancement of the digestive efficiency and modulation of</u> <u>energetic homeostasis</u>
- vitamin synthesis
- competitive barrier against colonization/invasion
- development, education and function of the immune system
- strengthening of the GIT epithelium impermeability
- detoxification of xenobiotics

And more recently:

- central nervous system modulation
- endocrine system modulation



HOW DO WE KNOW THIS?

- Microbiome sequence analysis
- Observation of human gut microbiota in different physiological/pathological condition
- In vitro studies using complex bacterial communities

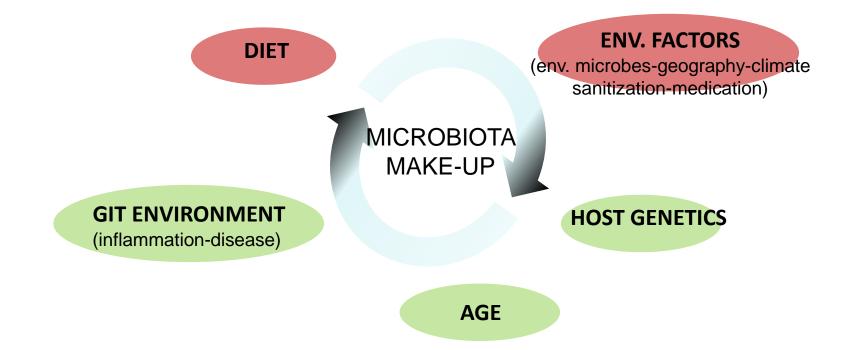


THE ADOPTION OF GERM-FREE MICE ALLOWED TO MEASURE THE IMPACT OF GUT MICROBIOTA-HOST MUTUALISM ON SEVERAL PHYSIOLOGICAL PARAMETERS



MICROBIOTA PLASTICITY

THE INDIVIDUAL MICROBIOTA COMPOSITION CONTINUOUSLY CHANGES IN RESPONSE TO **EXTRINSIC** AND **INTRINSIC** VARIABLES

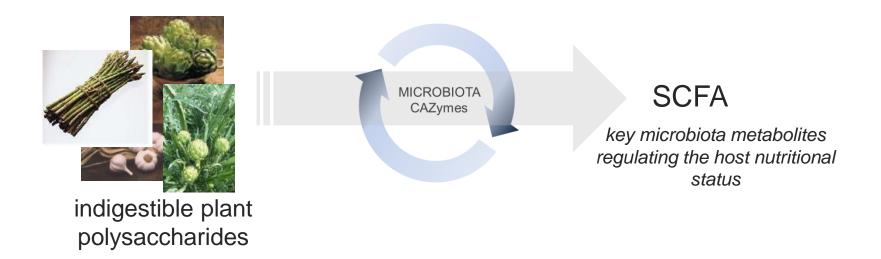


IN A MUTUALISTIC CONTEXT, THE PLASTICITY OF THE HUMAN MICROBIOTA GUARANTEES A RAPID ADAPTATION OF THE SUPER-ORGANISM IN RESPONSE TO DIET CHANGES, AGE, ETC there is a strong selection towards a readily changeable individual microbiome profile



IMPACT ON HOST NUTRITION

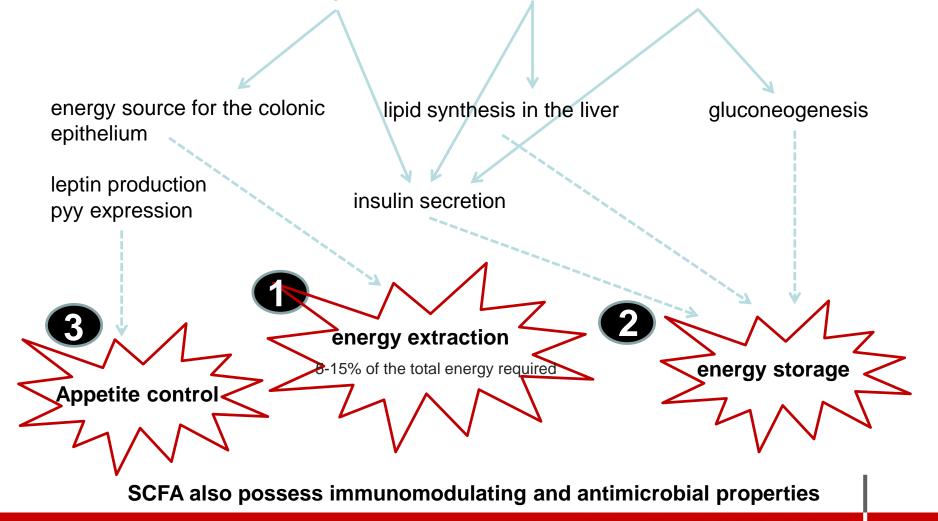
indigestible plant polysaccharides (xylan, pectin, arabinose containing-dietary carbohydrates as plant-derived pectin, cellulose, hemicellulose, resistant starches) reach unchanged the colon where they are metabolized by the intestinal microorganisms



equipped with a real arsenal of CAZymes – absent in human genome – intestinal microorganisms degrade plant polysaccharides to SCFA

SCFA, MICROBIAL METABOLITES WITH A KEY MULTIFACTORIAL ROLE IN HOST NUTRITION

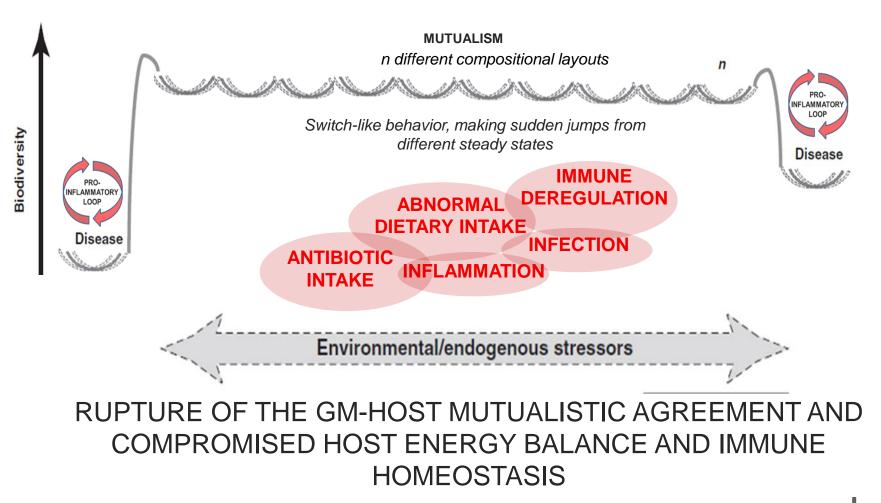
SCFA: butyrate - acetate - propionate



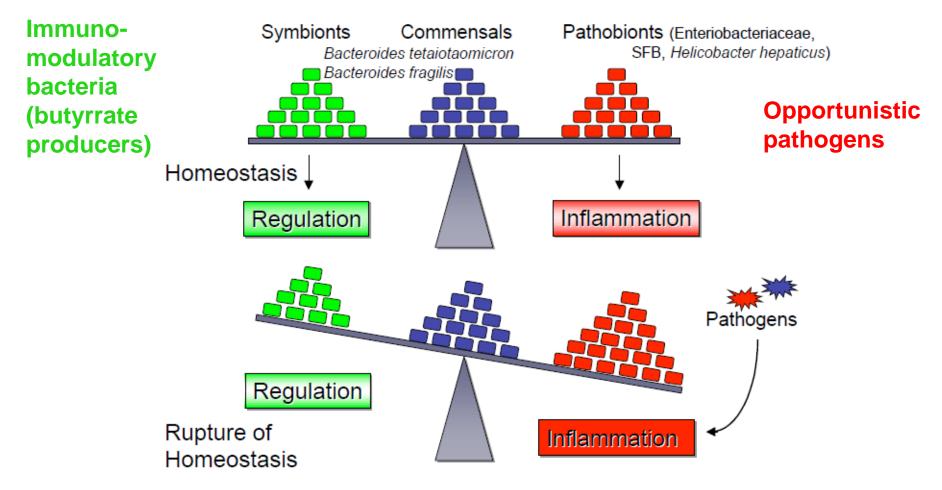


MUTUALISM BREAKDOWN

the GM is a multistable system with a variable fraction at 40% of the total community



IMMUNOLOGICAL DISREGULATION ASSOCIATED WITH MICROBIOTA DYSBIOSIS

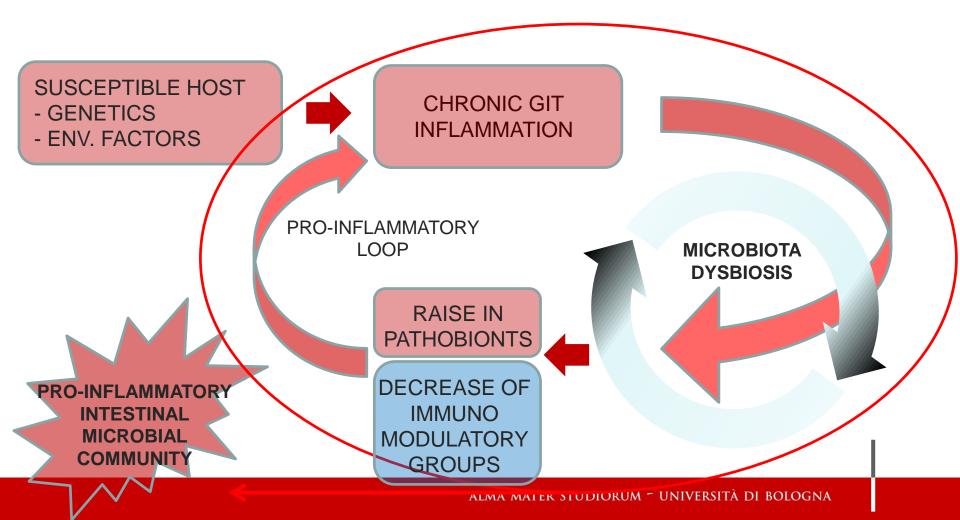


From Round JL & Mazmanian SK. 2009. Nat.Rev.Immunol.,9:313-323



INFLAMMATION AND MICROBIOTA

A non-controlled pro-inflammatory pathway can dramatically impact on the composition of the intestinal microbiota





GUT MICROBIOTA-ASSOCIATED DISORDERS

- Inflammatory bowel diseases Peterson et al. Cell Host and Microbe, 2008
- **Obesity** Turnbaugh PJ et al. Nature, 2006 (Increase in energy-harvesting bacterial populations)
- **Diabetes (type 1, 2)** Brugman S et al. Diabetologia, 2006, Amar et al. Am.J.Clin.Nutr., 2008 (Carbohydrate intake and glycemic control, insulin resistance)
- **Cardio-vascular diseases** (+ cholesterol dysregulation) Bäckhed F. Clin.Exp.Immunol., 2010
- Atopy/allergy Isolauri et al. Curr. Pharm. Des., 2008
- Colorectal Cancer: Wu et al. Nature Med., 2009 (chronic inflammation)
- Gut Brain Axis (IBS, depression, autism)



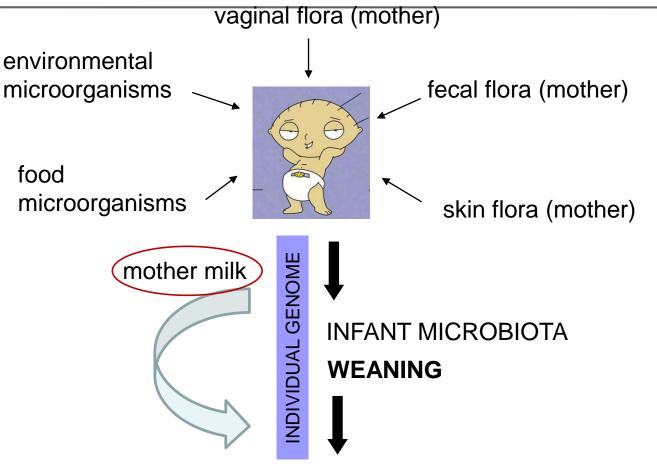


AGE RELATED CHANGES OF THE GUT MICROBIOTA PROVIDE THE HOST WITH **ECOLOGICAL SERVICES CALIBRATED FOR EACH STAGE OF LIFE**

Candela et al., Critical Rev Microbiol, 2013



WE ARE BORN STERILE IN A MICROBIAL WORLD



ADULT INDIVIDUAL MICROBIOTA (from 3rd year to all life)



INFANT-TYPE MICROBIOTA: SIMPLE, READILY CHANGEABLE AND BIFIDOBACTERIUM-DOMINATED

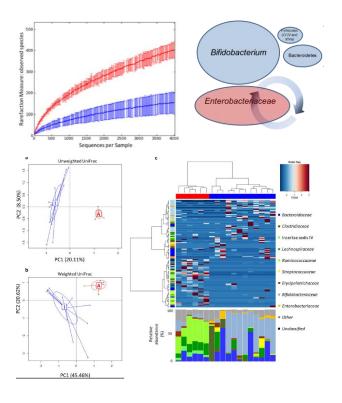


Table 3. TNF- α impact on the HT29 cell-associated microbiota fraction in breast-fed infants and adults.

	Breast-fed infants			Adults			
Microbial group	- TNF-α	+ TNF-α	FRD	- TNF-α	+ TNF-α	FRD	
Bacteroides - Prevotella	10.8	9.3	n.s.	23.5	15.4	0.008	
Clostridium cluster IV	1.7	2.5	0.520	21.5	18.2	n.s.	
Clostridium cluster IX	5.4	5.1	n.s.	5.2	3.4	n.s.	
Clostridium cluster XIVa	5.0	4.8	n.s.	28.7	37.5	0.154	
Clostridium cluster XI	0.5	0.6	n.s.	0.3	1.3	<0.001	
Clostridium cluster I, II	1.4	1.3	n.s.	1.2	1.8	0.24	
Lactobacillaceae	6.9	7.4	n.s.	1.9	2.1	n.s.	
Bifidobacteriaceae	14.4	13.1	n.s.	4.0	5.8	n.s.	
Verrucomicrobiae	0.8	1.0	n.s.	3.3	1.5	n.s.	
Bacillaceae	5.5	6.3	n.s.	1.8	4.0	0.004	
Fusobacteriaceae	0.6	0.8	0.570	1.1	1.7	0.132	
Enterococcales	10.9	10.4	n.s.	1.6	2.5	0.430	
Enterobacteriaceae	35.3	36.8	n.s.	5.5	3.8	0.360	
Campylobacteriaceae	0.7	0.8	n.s.	0.3	1.1	<0.001	

presence of environmental aerobes

INFANT-TYPE MICROBIOTRA IS STRUCTURED TO COPE WITH INFLAMMATION, BEING CO-EVOLVED TO PRIME THE EARLY IMMUNE SYSTEM IN THE CONTEXT OF TRANSIENT INFLAMMATORY RESPONSES; MILK DIGESTION; FOLATE BIOSYNTHESIS

Centanni et al., PLoS ONE. 2013



FECAL MICROBIOTA OF BREAST-FED INFANTS VS ADULTS

	Relative abundance	e (mean, %)	
Phylum	Breast-fed infants	Adults	*P value
Actinobacteria	46.0	7.6	0.024
Firmicutes	28.7	76.3	0.007
Proteobacteria	3.5	0.4	0.003
Genus			
Bifidobacterium	45.4	7.5	0.049
Alistipes	0	1.3	<0.001
Streptococcus	2.8	0.2	0.007
Blautia	0.04	6.6	<0.001
Lachnospiraceae	2.1	5.7	0.041
Roseburia	0.03	6.8	<0.001
Faecalibacterium	0.4	19.8	<0.001
Oscillibacter	0.01	1.5	<0.001
Ruminococcacea	e 0.3	3.8	0.002
Ruminococcus	0.03	8.7	0.011
Subdoligranulum	0.1	3.0	0.001
Veillonella	1.7	0.01	0.006
Erysipelotrichace	ae 0.3	2.8	0.004
Escherichia/Shige	ella 2.6	0.06	0.001



TNF-ALFA IMPACT ON THE HT29 CELL-ASSOCIATED MICROBIOTA FRACTION

	Breast-f	ed infants	Adults	
Microbial group	- TNF-α	+ TNF-α	- TNF-α	+ TNF-α
Bacteroides - Prevotella	10.8	9.3	23.5	15.4
Clostridium cluster IV	1.7	2.5	21.5	18.2
Clostridium cluster IX	5.4	5.1	5.2	3.4
Clostridium cluster XIVa	5.0	4.8	28.7	37.5
Clostridium cluster XI	0.5	0.6	0.3	1.3
<i>Clostridium</i> cluster I, II	1.4	1.3	1.2	1.8
Lactobacillaceae	6.9	7.4	1.9	2.1
Bifidobacteriaceae	14.4	13.1	4.0	5.8
Verrucomicrobiae	0.8	1.0	3.3	1.5
Bacillaceae	5.5	6.3	1.8	4.0
Fusobacteriaceae	0.6	0.8	1.1	1.7
Enterococcales	10.9	10.4	1.6	2.5
Enterobacteriaceae	35.3	36.8	5.5	3.8
Campylobacteriaceae	0.7	0.8	0.3	1.1

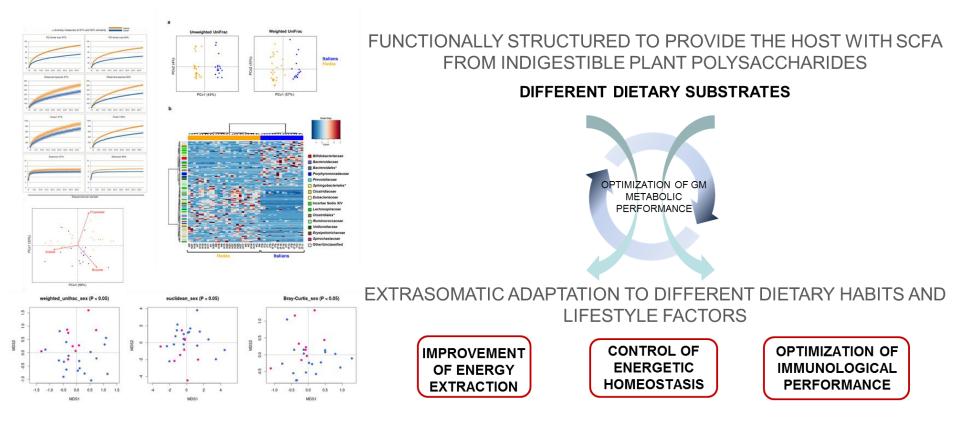


TAKE HOME MESSAGE

- The GM of breast-fed infants is selected for the establishment of a intense cross-talk with the host immune system
- Enriched in *Bifidobacterium* and *Enterobacteriaceae*, the infant-type microbiota is specifically structured to drive immune education in early infancy.
- Enterobacteriaceae provide genes associated with virulence functionality, which continuously boost the immunological response, whereas the bifidobacterial fraction dominated by the species *B. breve* and *B. longum* exert synergic immune modulatory and protective properties



ADULT-TYPE MICROBIOTA: COMPLEX AND ADAPTABLE ECOSYSTEM DOMINATED BY FIRMICUTES AND BACTEROIDETES



Schnorr et al., Nature Communication, 2014



CENTENARIAN GUT MICROBIOTA

Phylum/order	Genus-like phylogenetic group	Relative contribution (%) ^A			Ratio ^B		P value ^C	
		c	E	Y	C/E	C/Y	C vs E	C vs Y
Clostridium cluster XV	Eubacterium limosum et rel.	0.35	0.02	0.02	16	14.5	0.0009	0.01
Proteobacteria	Klebsiella pneumoniae et rel.	0.17	0.03	0.02	5.3	6.7	0.002	0.0009
	Vibrio	0.15	0.03	0.03	5.4	5.4	<0.0001	< 0.0001
	Enterobacter aerogenes et rel.	0.05	0.03	0.02	1.9	2.1	0.03	0.04
Actinobacteria	Eggerthella lenta et rel.	0.11	0.06	0.04	1.8	2.7	0.02	0.0001
Bacilli	Bacillus	0.07	0.05	0.03	1.4	2.0	0.01	0.04
Gostridium cluster №	Clostridium leptum et rel.	2.37	1.27	1.33	1.8	1.8	0.006	0.005
	Sporobacter termiditis et rel.	1.14	0.75	0.70	1.5	1.6	0.05	0.04
	Anaerotruncus colihominis et rel.	0.99	0.68	0.66	1.4	1.5	0.08	0.01
	Clostridium orbiscindens et rel.	1.52	1.05	1.16	1.4	1.3	0.03	0.08
	Faecalibacterium prausnitzii et rel.	2.01	4.05	4.24	0.5	0.5	0.01	0.006
	Papillibacter cinnamovorans et rel.	1.30	1.72	1.80	0.7	0.7	0.06	0.04
Clostridium cluster XIVa	Clostridium colinim et rel.	0.90	1.98	1.57	0.4	0.6	0.06	0.05
	Clostridium sphenoides et rel.	0.97	1.92	1.50	0.5	0.6	0.0002	0.003
	Eubacterium hallii et rel.	3.16	4.75	5.76	0.7	0.5	0.03	0.004
	Eubacterium rectale et rel.	1.68	3.61	3.02	0.5	0.5	0.001	0.004
	Eubacterium ventriosum et rel	1.21	2.77	2.62	0.4	0.4	0.0005	0.0002
	Lachnobacillus bovis et rel.	1.15	1.98	1.46	0.6	0.8	0.007	0.03
	Outgrouping Clostridium duster XIVa	0.63	0.94	1.04	0.7	0.6	0.02	0.01
	Roseburia intestinalis et rel.	1.57	3.04	3.21	0.5	0.5	0.006	0.03
	Ruminococcus lactaris et rel.	0.65	1.07	0.87	0.6	0.7	0.002	0.01
	Ruminococcus obeum et rel.	1.73	2.79	2.65	0.6	0.6	0.003	0.01

LOW DIVERSITY

DFCRFASE IN BIFIDOBACTERIA **SUBDOMINANT INCREASE IN PATHOBIONTS FRACTION** (Fusobacteria, Bacillus, Staphylococcus, Enterobacteriaceae) DOMINANT **REARRANGEMENT OF BUTYRATE FRACTION PRODUCERS** GM

> Anaerotruncus colihominis (CLIV) Eubacterium limosum (CIXV)

Pelative contributions of genux-like phylogenetic group to the fecal microbiota was calculated as percentage of signal intensities the total signal intensity. Patal of the average relative abundance of each genux-like phylogenetic group calculated for subjects belonging to C and E groups, and to C and Y groups. Bacterial groups showing (C and CY ratio - V decreased in the subjects of groups C. In tails, nelwant groups with P values ranging from 0.05 to 0.08 (tailscs) are reported. doi:01.217/juma.pne.010667.002

Ruminococcus obeum (CI XIVa) Roseburia intestinalis (CI XIVa) Eubacterium rectale (CI XIVa) Faecalibacterium prausnitzii (CLIV)

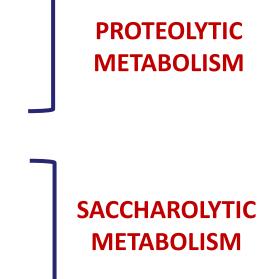
Biagi et al., PLos ONE, 2010



CENTENARIAN GUT MICROBIOME

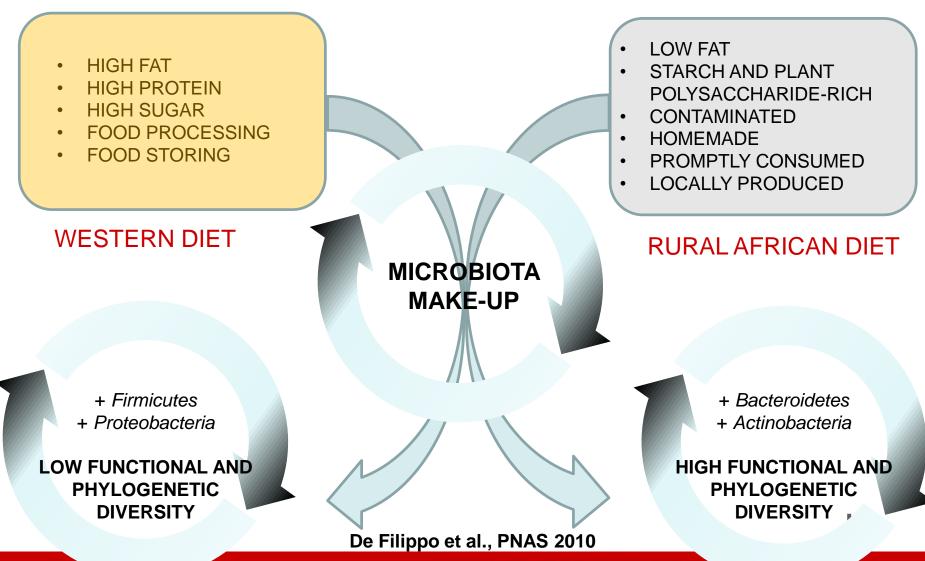
CENTENARIAN MICROBIOME IS ENRICHED IN GENES INVOLVED IN THE METABOLISM OF AROMATIC AMINO ACIDS (tryptophan and phenylalanine) AS WELL AS TYROSINE, VALINE AND LYSINE

ELDERLY AND YOUNG ADULT MICROBIOME IS ENRICHED IN GENES INVOLVED IN CARBOHYDRATE METABOLISM, PYRUVATE PRODUCTION AND AMINO ACID BIOSYNTHESIS





WESTERN vs RURAL DIET





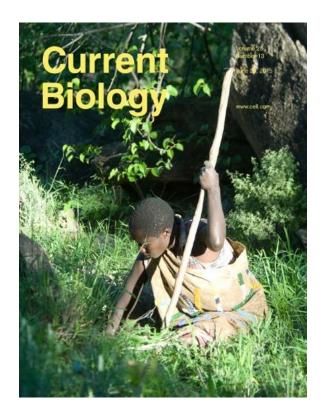
Current Biology

Volume 25, Issue 13, 29 June 2015, Pages 1682–1693

Article

Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota

Simone Rampelli¹, Stephanie L. Schnorr^{2,} ▲, ➡, Clarissa Consolandi³, Silvia Turroni¹, Marco Severgnini³, Clelia Peano³, Patrizia Brigidi¹, Alyssa N. Crittenden⁴, Amanda G. Henry², Marco Candela^{1,} ▲, ➡



Hazda lifestyle closely resembles that of paleolithic humans (no cultivation or domestication of plants and animals)

Ce



THE HADZA DIET

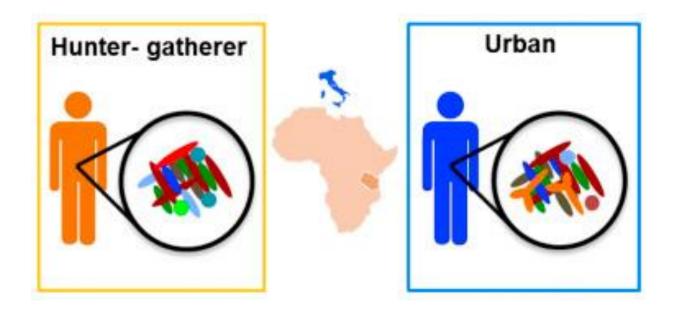


Wild foods consumed by Hazda: meat, honey, baobab, berries and tubers (no dairy foods)

Annual Hazda diet: 70% of kcal from plant foods; 30% from bird and animal meat (dry season) diet rich in simple sugars, starch and protein while lean in fat



SHOTGUN SEQUENCING OF TOTAL FAECAL DNA FROM 27 HADZA HUNTER-GATHERERS (AGE 8-70 Y) AND 16 ITALIANS (AGE 20-40 Y)



A total of 448.4 millions of 2x100 paired-end reads (11.8 millions \pm 1.7 per subject) were obtained

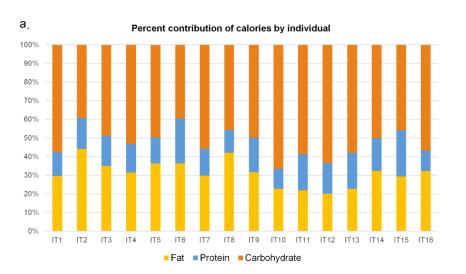
Schnorr et al., Nat Commun 2014

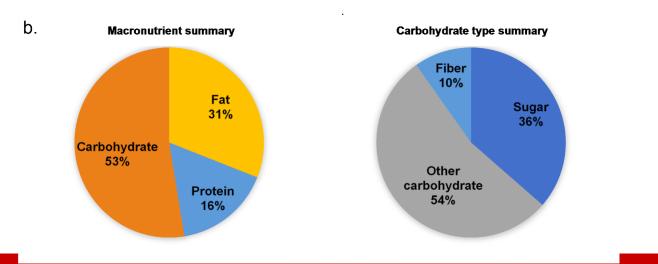


DIET OF THE ITALIAN COHORT

Mediterranean diet

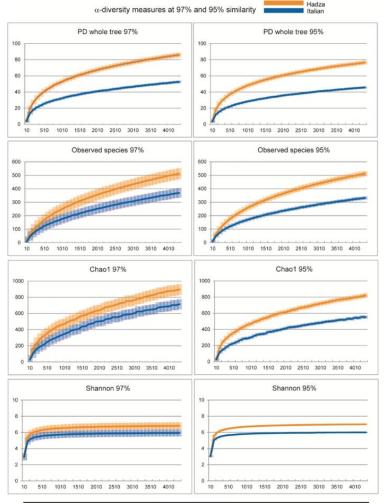
- abundant plant food, fresh fruit, pasta, bread and olive-oil;
- low to moderate amounts of dairy, poultry, fish and read meat







BIODIVERSITY OF THE HADZA MICROBIOTA



Sequences per sample

 α - diversity at 97% and 95% similarity

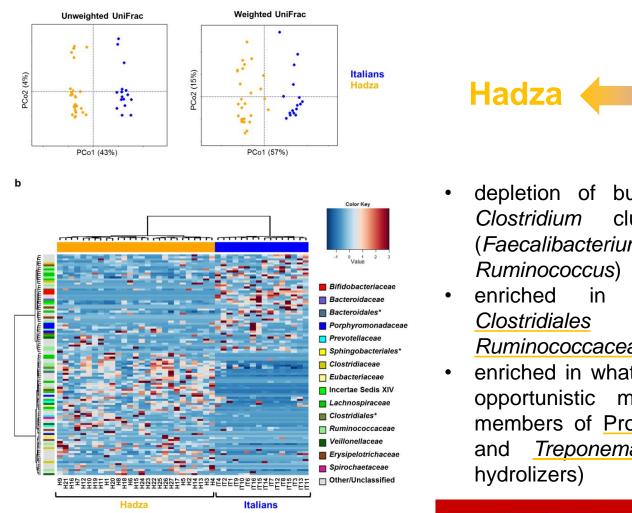
Hadza have a more diverse gut microbe ecosystem compared to the Italian cohort.



a

COMPARISON TO ITALIAN CONTROLS

Remarkable differences in subdominant phyla (< 10% relative abundance)



 depletion of butyrate producers of the Clostridium clusters IV and XIVa (Faecalibacterium, Roseburia,

Italians

- enriched in <u>Prevotella</u>, <u>unclassified</u> <u>Clostridiales</u> and <u>unclassified</u> <u>Ruminococcaceae</u>
- enriched in what are generally considered opportunistic microorganisms, such as members of Proteobacteria, <u>Succinivibrio</u>, and <u>Treponema</u> (cellulose and xylan hydrolizers)



ABSENCE OF *BIFIDOBACTERIUM* IN HAZDA

Never reported for any other human group (confirmed by qPCR)

Hypothesis

- Absence of agro-pastoral-derived foods (consumption of dairy into adulthood could in most Western populations maintains a relatively large bifidobacteria presence in post weaning GM composition)
- Hadza neither domesticate nor have direct contact with livestock animals (swine, cattle and rabbit) → conditions for interspecies transfer and colonization of bifidobacteria do not occur

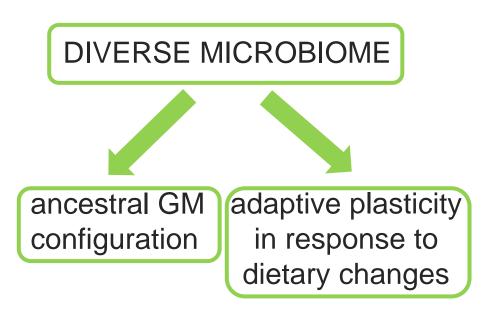
Absence of bifidobacteria combined with the enrichment in "opportunistic" bacteria (Proteobacteria and Spirochaetes)

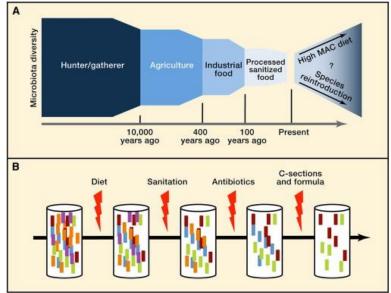
Hadza GM likely represents a new equilibrium that is beneficial and symbiotic to their living environment



DIVERSITY, THE UNIVERSAL MARKER FOR A HEALTHY MICROBIOME

disease associated-GM dysbioses always involves a reduction of ecosystem diversity





Sonnenburg E, Sonnenburg J, Cell Metabolism 2014

The transition from rural communities to modern urban societies involve the progressive loss of gut microbiome species

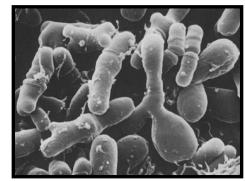


MANIPULATION OF THE HUMAN INTESTINAL MICROBIOTA

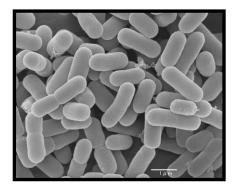


PROBIOTICS

A LIVE MICROBIAL FEED SUPPLEMENT WHICH BENEFICIALLY AFFECTS THE HOST



PROBIOTICS SHOULD BE ABLE TO SURVIVE AND MULTIPLY IN THE HOST GI TRACT BUT RAPIDLY DISAPPEAR WHEN ORAL ADMINISTRATION IS STOPPED



USE: FUNCTIONAL FOODS

(FERMENTED MILKS, INFANT FORMULA, FRUIT DRINKS)

PHARMACEUTICAL PREPARATION

DRIED SUPPLEMENTS

lma mater studiorum - università di bologna



PROBIOTICS SELECTION CRITERIA

For a microorganism to be characterized as *probiotic* a number of criteria have to be met:

- Non-pathogenic and non-toxic
- Beneficial effect on the consumer
- Survival in the gastrointestinal tract
- Preferably isolated from the same species as the intended use



MOST COMMONLY USED PROBIOTICS

LACTIC ACID BACTERIA

LACTOBACILLUS (L. acidophilus, L. casei,L. delbrueckii,L. gasseri, L. rhamnonsus, L. cellobiosus, L. curvatus, L. fermentum, L. lactis, L. plantarum, L. reuteri, L. salivarius, L. brevis)

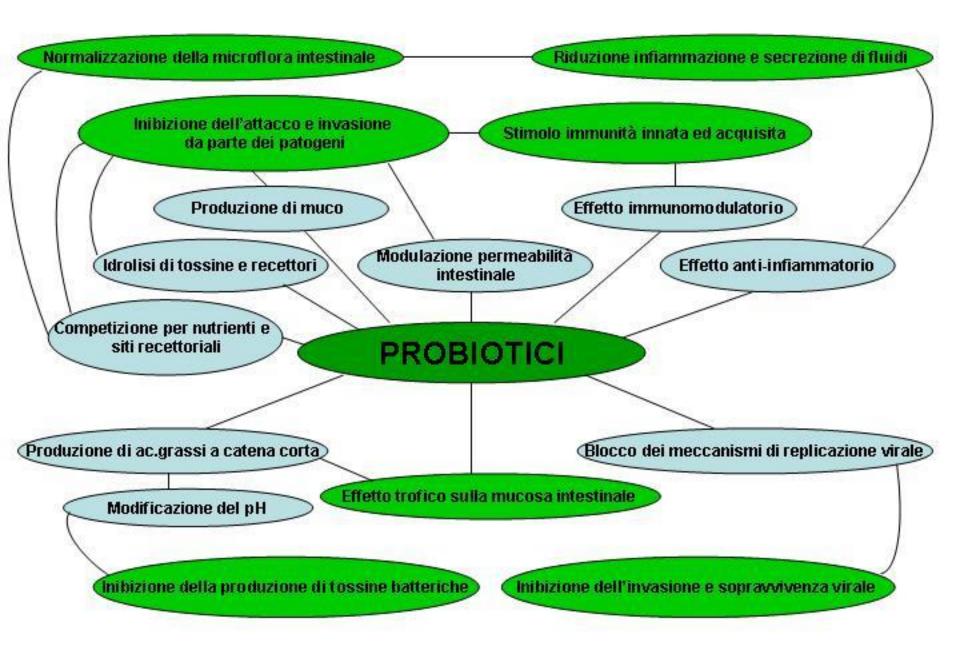
BIFIDOBACTERIUM (B. longum, B. bifidum, B. adolescentis, B. infantis, B. breve, B. animalis, B. lactis)

ENTEROCOCCUS (E. faecalis, E. faecium)

PROBIOTICS OTHER THAN LAB

BACILLUS SPORES (B. cereus, B. clausii, B. subtilis) SACCHAROMYCES (S. boulardii, S. cerevisiae)

HEALTH PROMOTING EFFECTS OF PROBIOTICS





APPLICATION OF PROBIOTICS: DISEASE'S PREVENTION AND TREATMENT

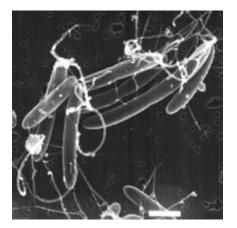
Cardiovascular disease Colon cancer **Diarrhea** (acute diarrhea, antibiotic-associated diarrhea, radiation-induced diarrhea) Allergy Hepatic encephalopathy Immune response **Inflammatory bowel disease (IBD**, Pouchitis, Ulcerative colitis, Crohn's disease) Irritable bowel syndrome (IBS) Lactose malabsorption **Necrotizing enterocolitis** Nonalcoholic fatty liver disease **Prevention of systemic infections**



NEXT GENERATION PROBIOTICS

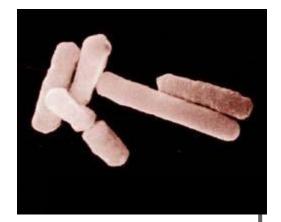
Faecalibacterium prausnitzii





Roseburia

Bacteroides

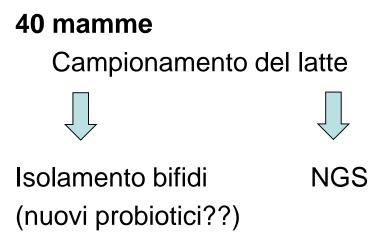






PROmozione della Salute del consumatore: valorizzazione nutrizionale dei prodotti agroalimentari della tradizione ITaliana





40 neonati Swab della bocca e feci





ONGOING STUDIES: FP7 KBBE PROJECTS



New dietary strategies addressing the specific needs of the elderly population for healthy ageing in Europe



Microbiome influence on energy balance and brain development/function put into action to tackle diet-related diseases and behaviour



THANKS FOR YOUR ATTENTION



ALMA MATER STUDIORUM ~ UNIVERSITÀ DI BOLOGNA

IL PRESENTE MATERIALE È RISERVATO AL PERSONALE DELL'UNIVERSITÀ DI BOLOGNA E NON PUÒ ESSERE UTILIZZATO AI TERMINI DI LEGGE DA ALTRE PERSONE O PER FINI NON ISTITUZIONALI