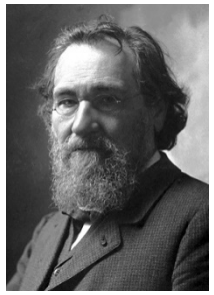
Patrizia Brigidi
Dipartimento Dipartimento di Farmacia e Biotecnologie
Università di Bologna

I probiotici: aspetti normativi, biologici e clinici

HISTORY OF PROBIOTICS

Fermented products used **as long as human history**

1857: Pasteur discover LAB



1908: Metchnikoff suggests health benefit of LAB

1935: Asia, first commercial LAB-containing product

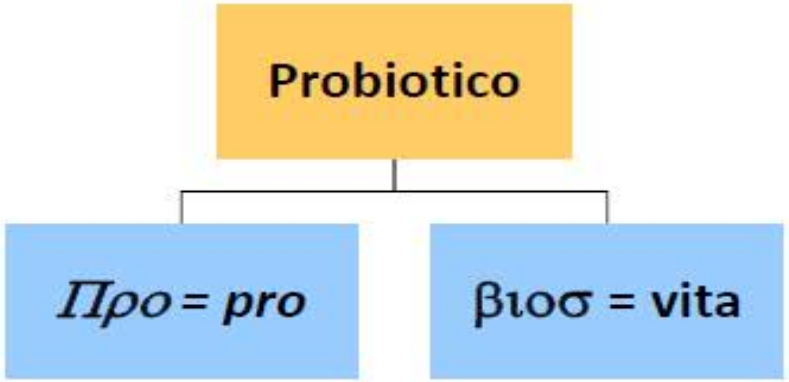
1965: First use of the term **PROBIOTIC**

1980's: Europe, first commercial probiotic yoghurts

2000 to now: increasing awareness of probiotic potential in consumers

1

PROBIOTICS DEFINITION



live microorganisms which when administered in adequate amounts confer a health benefit on the host

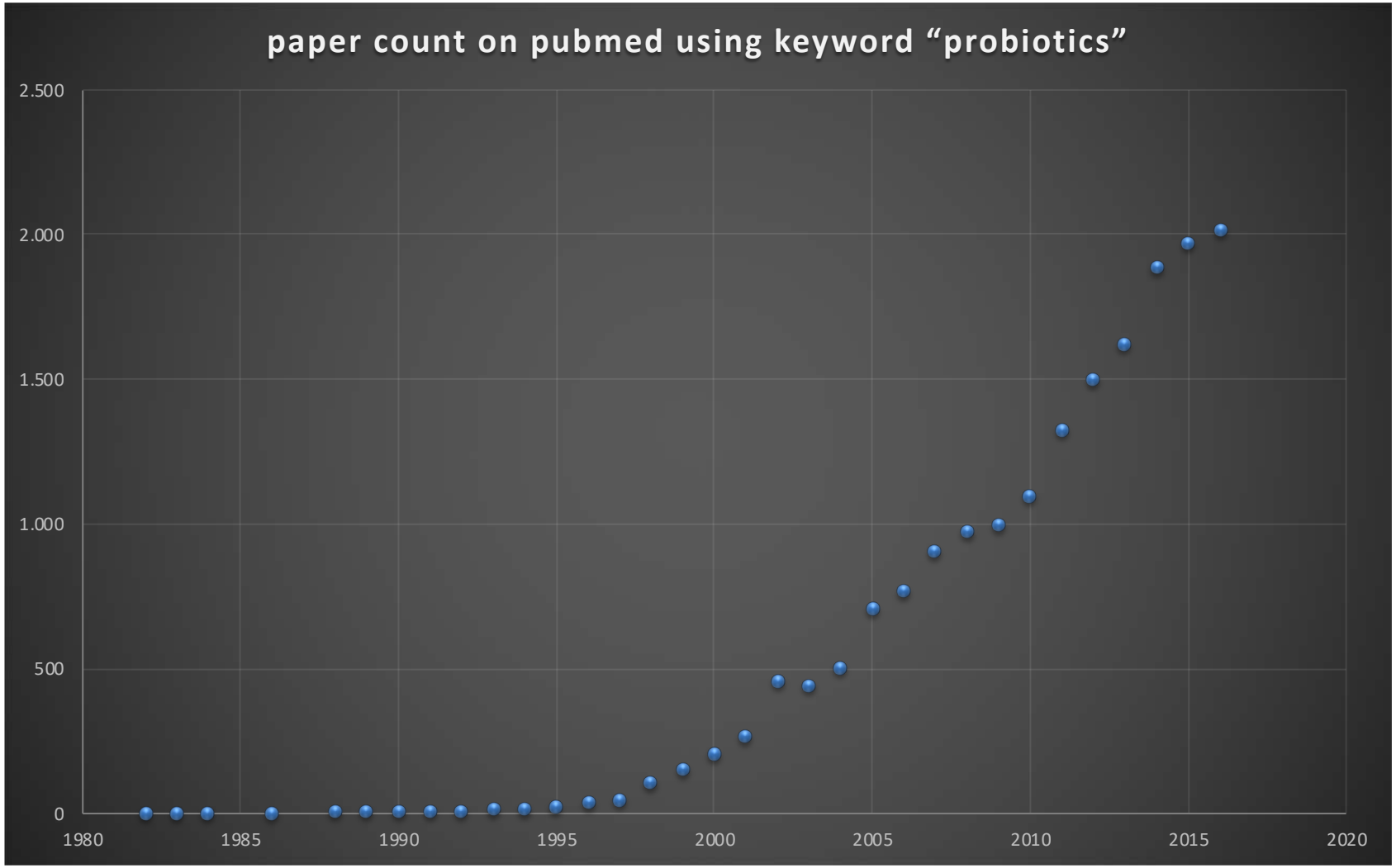
WHO (FAO/WHO), 2001

live microorganisms that, when administered in adequate amounts, confer a health benefit on the host

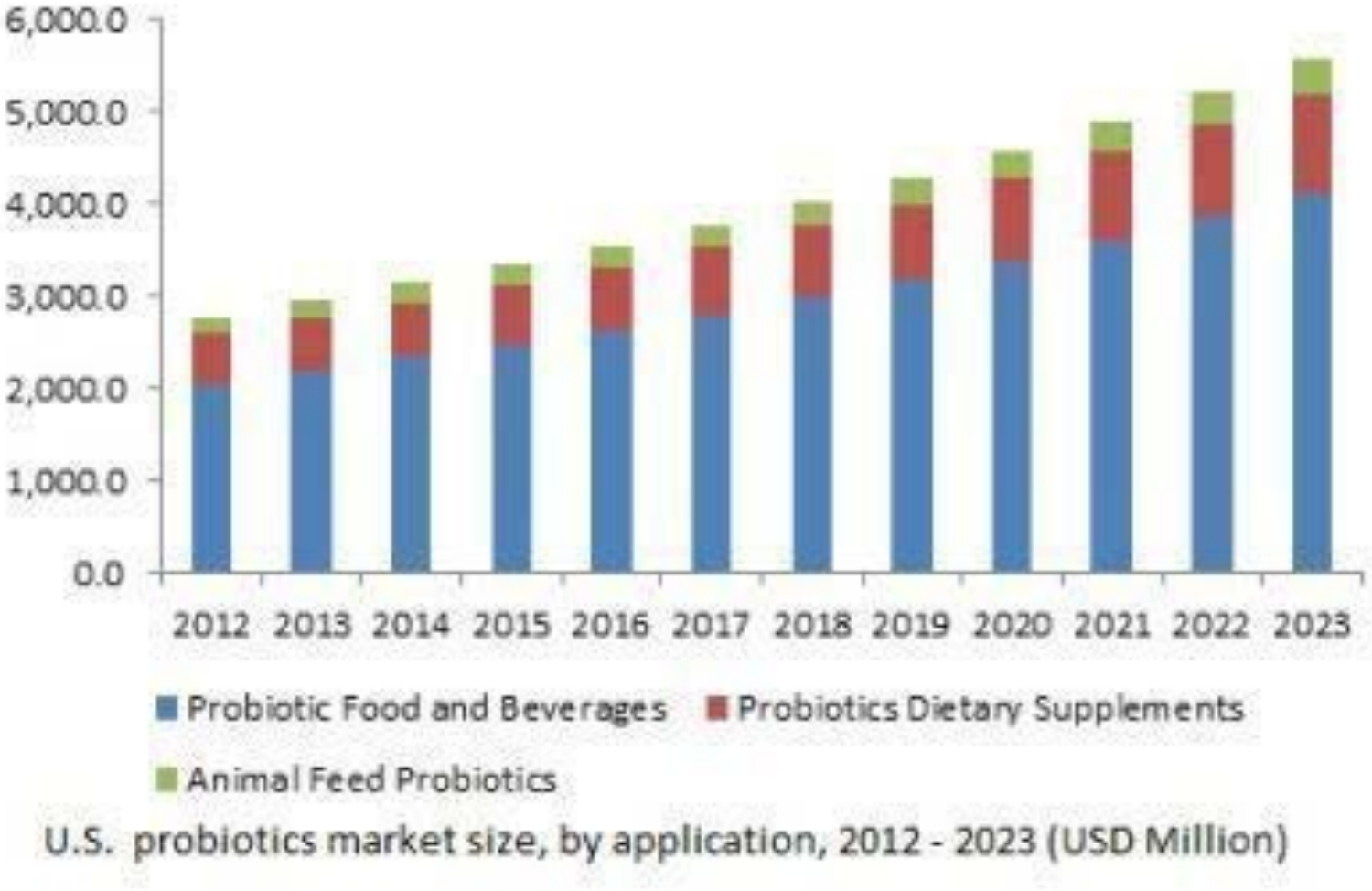
International Scientific Association for Probiotics and Prebiotics (ISAPP), 2014

Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

SCIENTIFIC PRODUCTIVITY IN THE FIELD

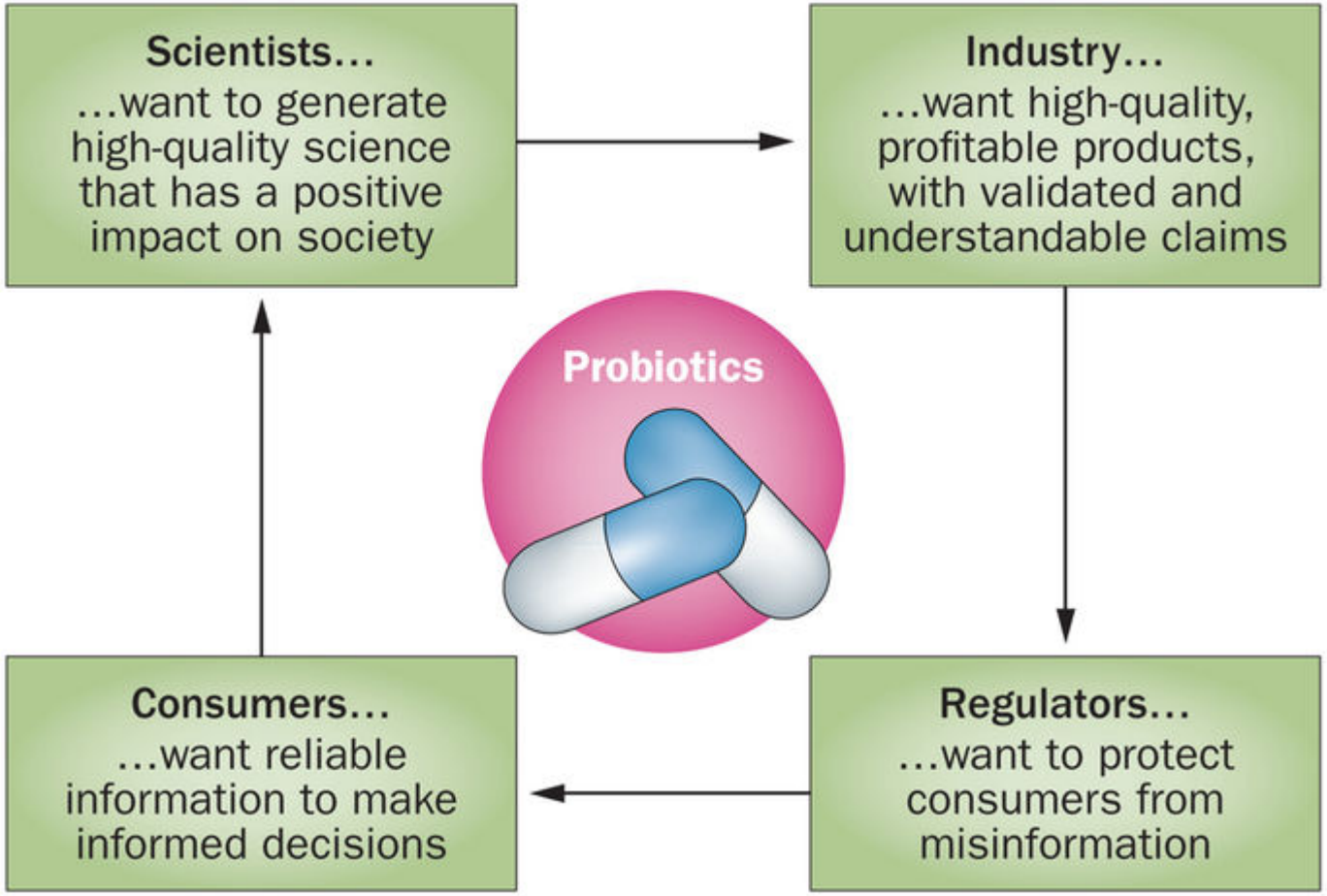


USA PROBIOTICS MARKET



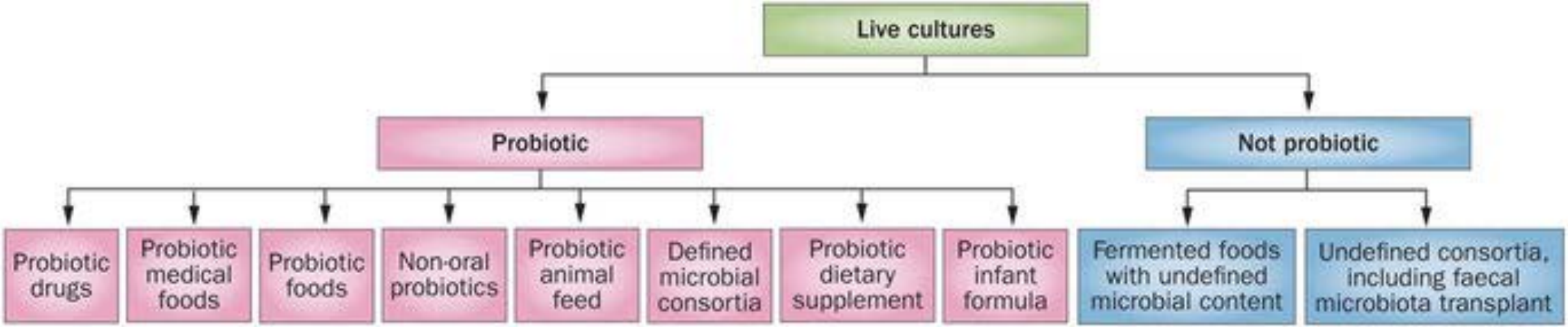
U.S. probiotics market size, by application, 2012 - 2023 (USD Million)

OBJECTIVES OF STAKEHOLDERS IN THE PROBIOTIC FIELD



Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

OVERALL FRAMEWORK FOR PROBIOTIC PRODUCTS



Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

MOST USED «PROBIOTIC» MICRORGANISMS

Bifidobacterium: *B. adolescentis, B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, *B. breve*, *B. bifidum*, *B. animalis* subsp. *lactis etc*

Lactobacillus: *L. gasseri*, *L. reuteri*, *L. acidophilus*, *L. casei*, *L. fermentum*, *L. johnsonii*, *L. rhamnosus*, *L. plantarum*, *L. paracasei*, etc.

Streptococcus thermophilus, Streptococcus salivarius

Propionibacterium spp.

Enterococcus faecium

***Bacillus* spores:** *B. cereus*, *B. clausii*, *B. subtilis, B. coagulans*

Saccharomyces boulardii

Escherichia coli Nissle

Food and dietery supplement dose:
10^9 / serving

SAFETY

Most probiotic microorganisms are GRAS
Few systemic safety studies in vulnerable population (infants, children, elderly, hospitalized children, hospitalized adults, immunocompromised, pregnant women,….)

Pathogenicity
Infectivity
Toxicity
Antibiotic-resistance (conjucation transfer)

GENOME SEQUENCING

INDUSTRIAL GROWTH AND STABILITY

Inoculum, substrates, oxygen level, temperature, pH, ……..
Food additives and ingredients, processing and storage

COMMON PROBIOTIC TRAITS

GENOME SEQUENCING

- Adhesion to the host
- Efflux systems to survive gastric environment
- Hydrolases to confer bile-salt tolerance
- Metabolism optimized for conversion of carbohydrates to lactic acid and, in some cases, a mixture of other acids
- Rapid acidification and low-to-moderate growth yield

3

INGESTED BACTERIA – MEDIATED MICROBIOME

Ingested microbial cells:10^9

Firmicutes Bacteroidetes Proteobacteria Actinobacteria Other

	Taxonomy (phylum level)	Resident bacteria (number/ml or g)	Transit time[a]	Relative abundance of ingested bacteria compared to resident bacteria[b]
Stomach[c]		10^2–10^4	15 min–3 h	100 to 10 000-fold
Small intestine (ileum)[d]		10^6–10^8	2–5 h	0.01 to 1-fold
Colon (feces)[e]		10^{10}–10^{11}	12–24 h	0.0001 to 0.00001-fold

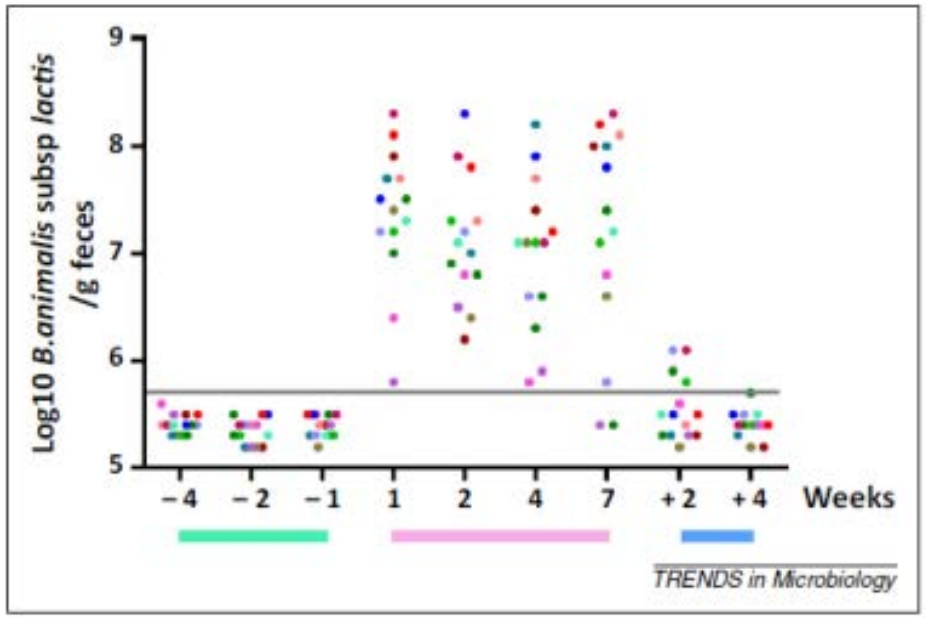
Derrien and Vlieg, 2015, Trends Microbiol

FATE OF INGESTED STRAIN

«PERSISTENCE» = fecal quantification of the ingested strain, reflecting the extent of cell death and subsequent replication of surviving cells

Strain dependent

High inter-individual variability (strains are rarely detected after 1 w)



Preconsumption period

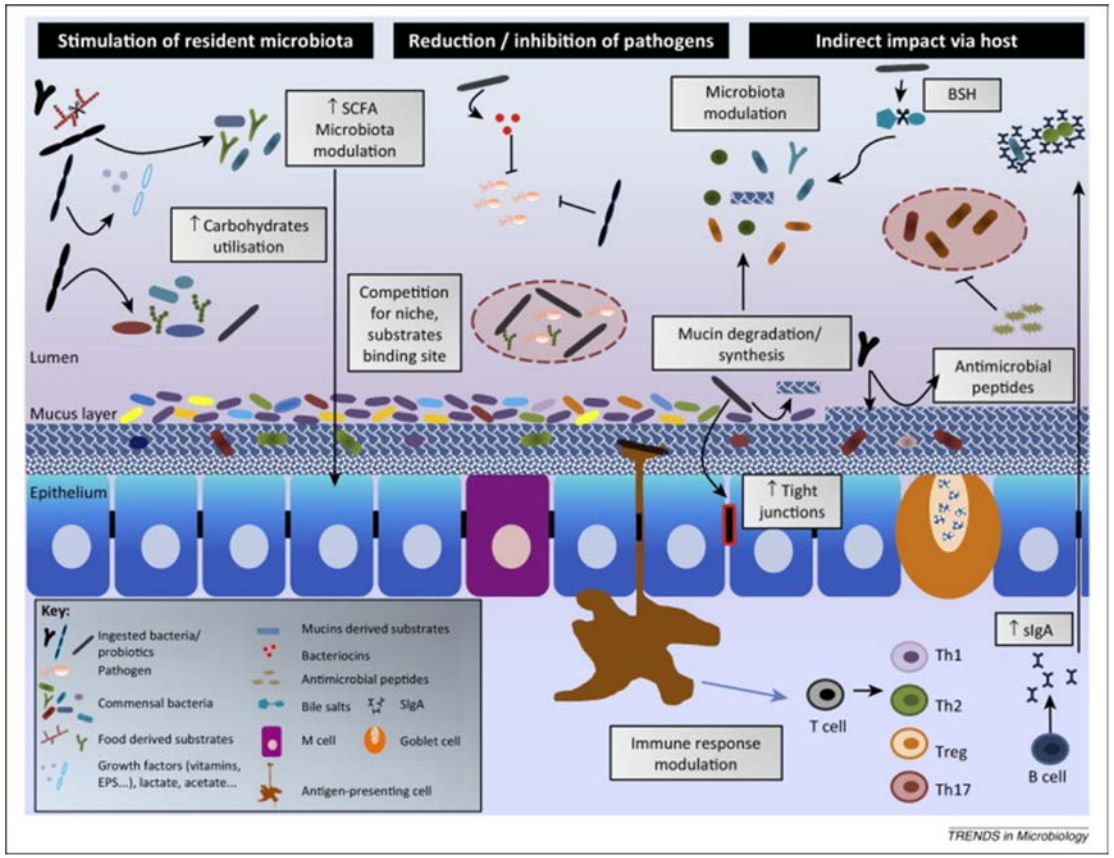
Consumption period

Postconsumption period

Derrien and Vlieg, 2015, Trends Microbiol

5

TRANSIENT INTEGRATION IN THE GUT MICROBIOTA ("TRANSIENT MICROBIOTA")

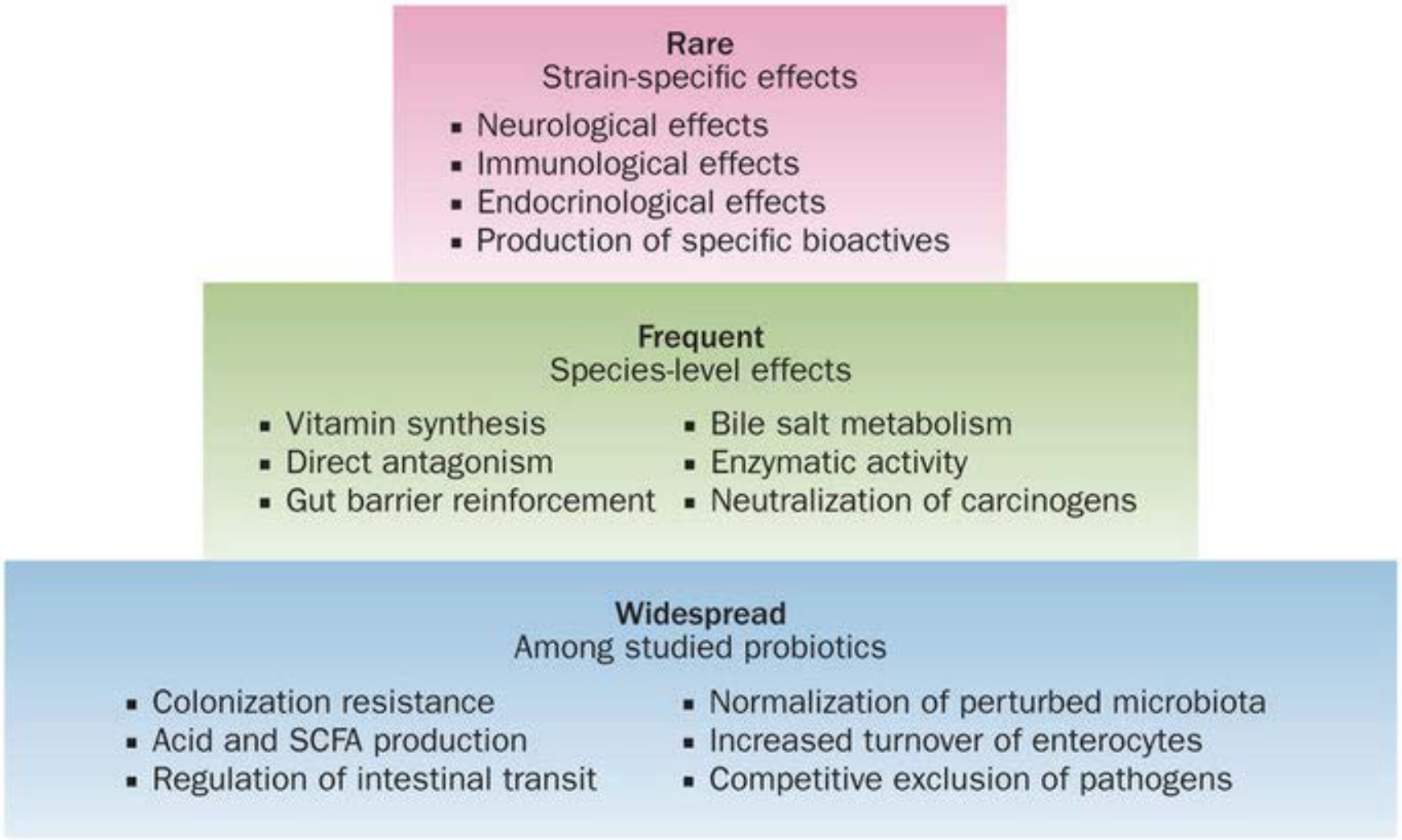


TRANSIENT MICROBIOME CAN IMPACT ON COMPOSITION AND ACTIVITY OF THE RESIDENT COMMUNITY:

- ✓ ***Stimulation of resident microbiota by trophic interaction*** *(metabolites, growth factors, carbohydrate metabolism, mucin degradation)*
- ✓ ***Reduction/inhibition of pathogens*** *through alteration of the microbial fitness (**pH decrease, niche competition, EPS and bacteriocins**)*
- ✓ *Indirect impact via host through **changes in the gut environment** (mucin production, increase of IgA and defensins)*

Derrien and Vlieg, 2015, Trends Microbiol

POSSIBLE DISTRIBUTION OF MECHANISMS AMONG PROBIOTICS



Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

ACCEPTED CORE BENEFITS OF CERTAIN PROBIOTICS

Large number of different probiotic strains belonging to commonly studied species

SUPPORTING A HEALTHY DIGESTIVE TRACT
beneficial in the treatment and prevention of GI diseases

NO SPECIFIC HEALTH CLAIM

ACCEPTED CHARACTERIZATION AT SPECIES LEVEL

no at strain level

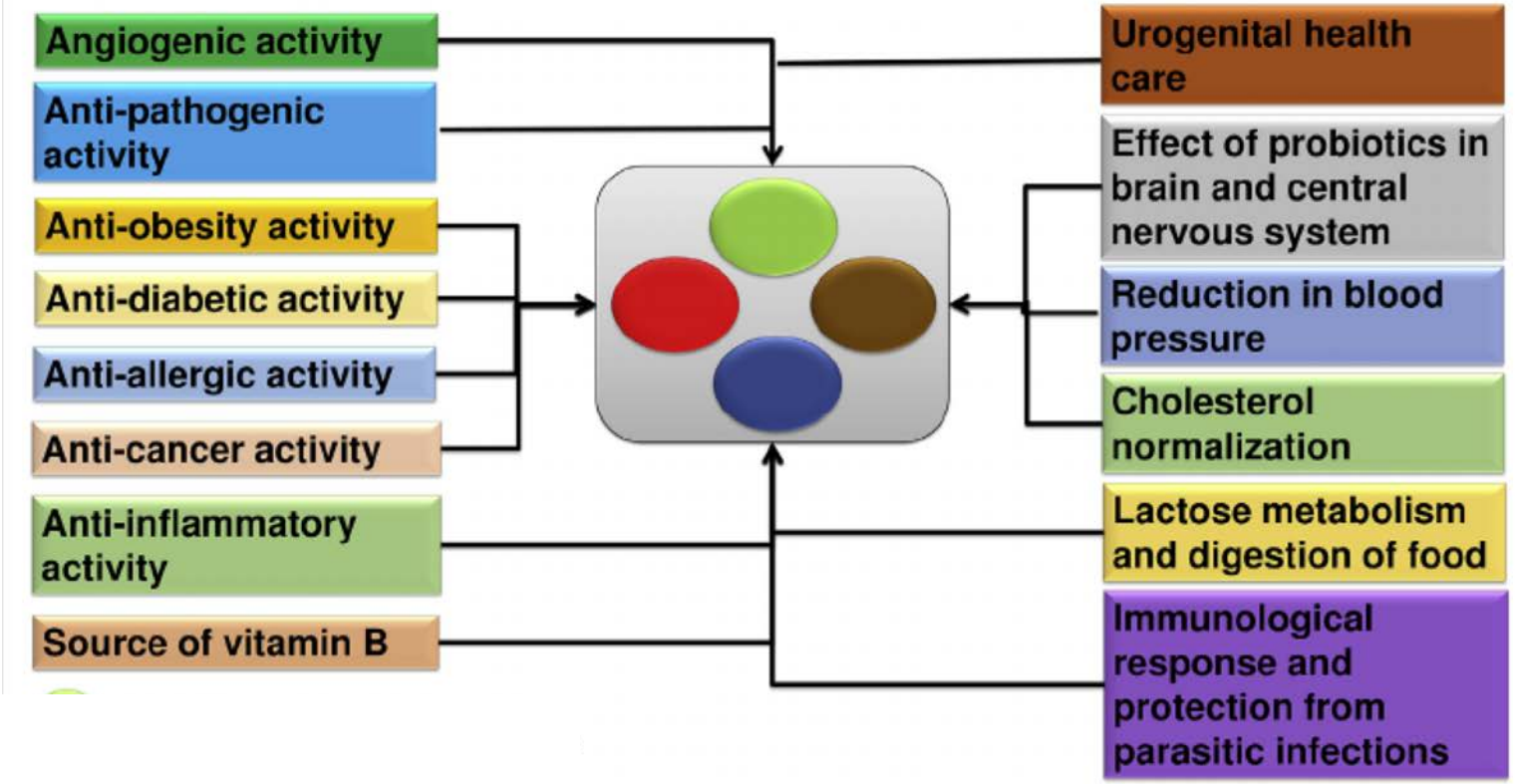
Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*

AIDING LACTOSE DIGESTION
mechanism of action: microbial production of beta-galactosidase

APPROVED CLAIM

APPLICATIONS OF PROBIOTICS AND THEIR MODE OF ACTION

(strain specific)



Kerry GR, et al., J Food and Drug Anal, 2018

MEASURE OF THE PROBIOTIC EFFECTS: HERE COMES THE PROBLEM

STUDIES ON HUMAN

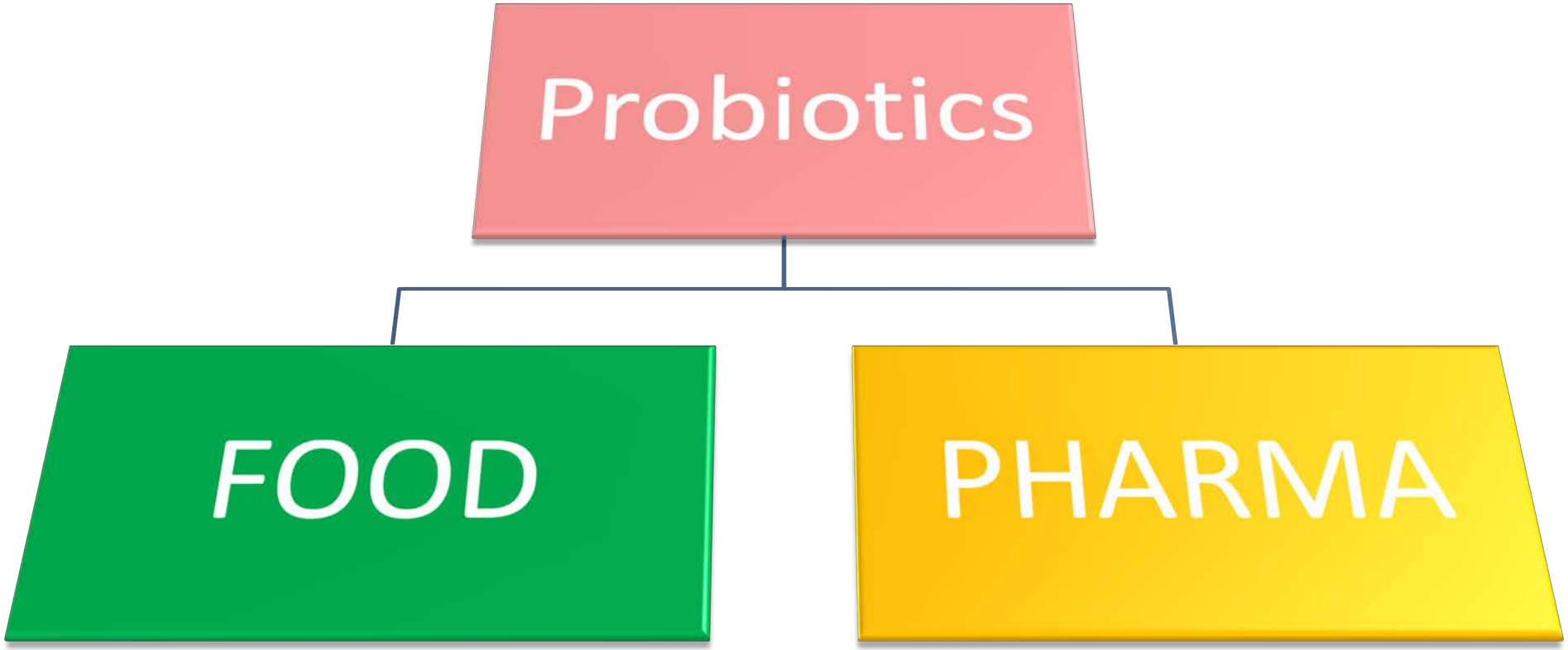
- **Heterogeneity of chosen strain (or mix of strains), duration of the intervention, dosage**
- **Heterogeneity of matrix (dairy food, capsule, powder…)**
- **Study design (comparison with placebo or baseline)**
- **Chosen population (adults, children, elderly; health, disease)**
- **Evaluation of the effects on health (long term, short term; therapeutic, preventive; …)**
- **Evaluation of the effect on the resident microbiota (different techniques)**

Derrien and Vlieg, 2015, Trends Microbiol

STRAINS	COMMERCIAL PRODUCTS	SOURCE
Lactobacillus acidophilus NCFM Bifidobacterium lactis HN019 (DR10)Lact. rhamnosus HN001 (DR20)	Sold as ingredient	Danisco (Madison, WI)
Saccharomyces cerevisiae boulardii	Florastor	Biocodex (Creswell, OR)
Bifidobacterium infantis 35,264	Align	Procter and Gamble (Mason, OH)
Lactobacillus fermentum VRI003 (PCC)	Sold as ingredient	Probiomics (Eveleigh, Australia)
Lactobacillus rhamnosus R0011Lactobacillus acidophilus R0052	Sold as ingredient	Institut Rosell (Montreal, Canada)
Lactobacillus acidophilus LA5Lactobacillus paracasei CRL 431	Sold as ingredient	Chr. Hansen (Milwaukee, WI)
Bifidobacterium lactis Bb-12	Sold as ingredient	Chr. Hansen (Milwaukee, WI)
Lactobacillus casei strain Shirota, Bifidobacterium breve strain Yakult	Yakult	Yakult (Tokyo, Japan)
Lactobacillus casei DN-114 001 (“L. casei Immunitas”) Bifidobacterium animalis DN173 010 (“Bifidis regularis”)	DanActive fermented milkActivia yogurt	Danone (Paris, France)Dannon (Tarrytown, NY)
Lactobacillus reuteri RC-14Lact. rhamnosus GR-1	Femdophilus	Chr. Hansens (Milwaukee, WI)Urex Biotech (London, Ontario, Canada)Jarrow Formulas (Los Angeles, CA)
Lactobacillus johnsonii Lj-1 (same as NCC533 and formerly Lactobacillus acidophilus La-1)	LC1	Nestlé (Lausanne, Switzerland)
Lactobacillus plantarum 299 V	Sold as ingredient; Good Belly juice product	Probi AB (Lund, Sweden); NextFoods (Boulder, Colorado)
Lactobacillus rhamnosus 271	Sold as ingredient	Probi AB (Lund, Sweden)
Lactobacillus reuteri ATCC 55,730 (“L. reuteri Protectis”)	BioGaia Probiotic chewable tablets or drops	Biogaia (Stockholm, Sweden)
Lactobacillus rhamnosus GG (“LGG”)	Culturelle; Dannon Danimals	Valio Dairy (Helsinki, Finland)The Dannon Company (Tarrytown, NY)
Lactobacillus rhamnosus LB21Lactococcus lactis L1A	Sold as ingredient	Essum AB (Umeå, Sweden)
Lactobacillus salivarius UCC118	–	University College Cork (Cork, Ireland)
Bifidobacterium longum BB536	Sold as ingredient	Morinaga Milk Industry Co. Ltd. (Zama-City, Japan)
Lactobacillus acidophilus LB	Sold as ingredient	Lacteol Laboratory (Houdan, France)
Lactobacillus paracasei F19	Sold as ingredient	Medipharm (Des Moines, Iowa)
Lactobacillus paracasei 33 Lact rhamnosus GM-020 Lact paracasei GMNL-33	Sold as ingredient	GenMont Biotech (Taiwan)
Lactobacillus plantarum OM	Sold as ingredient	Bio-Energy Systems, Inc. (Kalispell, MT)
Bacillus coagulans BC30	Sustenex, Digestive Advantage and sold as ingredient	Ganeden Biotech Inc. (Cleveland, Ohio)
Streptococcus oralis KJ3 Strept uberis KJ2 Strept rattus JH145	ProBiora3EvoraPlus	Oragenics Inc. (Alachua, FL)
Lactobacilli rhamnosus PBO1Lactobacilli gasseri EB01	EcoVag	Bifodan (Denmark), www.ecovag.com

Disease	Strains	Health impact	References
Hyper cholesteromia and cardiovascular diseases	*Lactobacillus spp., Bifidobacterium* spp., *Enterococcus faecium, Lactobacillus plantarum, Propionibacterium freudenreichii, Lactobacillus plantarum*	Positive; reduce the dietary cholesterol absorption	Suvarna and Boby, 2005; Parvez et al., 2006; Oxman et al. (2001); Homayouni et al. (2012); Sanders et al. (2005); Nguyen et al. (2013)
Diarrhoea	*Lb. rhamnosus, Lb. casei, Bf. lactis, Bf. Bifidum, Sc. Thermophilus, Lactobacillus casei*	Positive (Competition with pathogenic bacteria on epithelial cells)	Parvez, Malik, Ah Kang, and Kim (2006)
Antibiotic therapy	*Lb. salivarius, Lb. acidophilus, Lb. johnsonii, Enterococcus mundtii, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus* strains, *Bifidobacterium* strains	Positive (Minimize the disruptive effect of antibiotics to normal bacterial flora)	Sanders et al. (2005)
Kidney stones	*Lactobacillus RC−14, LactobacillusGR−1, Lactobacillus B−54*	Positive (Degrade or reduce the oxalate excretion)	Sanders et al. (2005)
Immunity	*Lb. casei Shirota, Lb. rhamnosus, Lb. acidophilus, Bf. lactis, Bacillus circulans, Lactobacillus plantarum*	Positive (Enhance the level of immune reactive cells)	Sanders et al. (2005); Homayouni et al. (2012)
Lactose intolerance	*Lb. rhamnosus, Lb. Plantarum, Lb. delberukii, Bf. Lactis, Lactobacillus acidophulus*	Positive(Digestion of lactose)	Sanders et al. (2005)
Cancer	*Bifidobacterium* sp., *Lb. casei Shirota, Lb. acidophilus, Propionibacterium sp, Lb. rhamnosus*	Positive (Detoxify the ingested carcinogens)	Sanders et al. (2005)
Hypertension	*Lb. Rhamnosus, Lb. Lactis*	Positive (Reduce blood pressure)	Sanders et al. (2005)
Pancreatitis	*Lb. rhamnosus GG, Bf. lactis BB−12*	Positive (Decrease in occurrence of pancreatic infection)	Pezzilli and Fantini (2006)
Tooth problems	Lactobacillus, Bifidobacterium	Positive, Decrease teeth problems	Darwazeh and Darwazeh (2011); Niers et al. (2009)
Anemia	Lactobacillus	lactobacilli increase the expression of iron transporters in the caecum due to production of propionic acid,	Balamurugan et al. (2010)
Eczema	*Escherichia coli, Bifidobacterium bifidum, Bifidobacterium lactis, Lactococcus lactis*	Eczema can be cured with probiotics	Niers et al. (2009); Soh et al. (2009); Viljanen and Pohjavuori (2005); Viljanen, Kuitunen et al. (2005)
Food allergies	*Escherichia coli, Lactobacillus, Bifidobacterium*	Improves immunity of body and reduces food allergies	Soh et al. (2009)
Urinary tract infection	*Lactobacillus rhamnosus, Lactobacillus reuteri, L. acidophilus*	Urinary tract diseases problems are reduced.	Anukam, Hayes, Summers, and Reid (2009)
Inflammatory Bowel diseases	*E. coli, Saccharomyces boulardii, Bifidobacterium longum, B. breve, B infantis, Lactobacillus casei, L. plantarum, L. acidophilus, L. delbrueki* subsp. *bulgaricus and Streptococcus salivarius* subsp. *thermophilus*	Bowel syndrome and inflammatory bowel disease are reduced	Ventura and Perozzi (2011)

Panghal et al., Food Bioscience, 2018


Probiotics
FOOD
PHARMA

DITTA	MEDICINALE	PRINCIPIO ATTIVO	FORMA FARMACEUTICA
Scharper	BACTOCIN	*L plantarum* I0^{8} UFC	Capsule mollli e soluzione vaginale
Sanofi	BIOFLORIN	Enterococco tipo L.A.B. ceppo SF68. 75 x 10^{6} UFC	Capsule rigide
Zambon	CODEX	*Saccharomyces boulardii* 5 x 10^{9} UFC	Capsule e polvere
Proge Medica	ECOCILLIN	*L plantarum*x 10^{9} UFC	Capsule molli vaginali
Sanofi	ENTEROGERMINA	Spore di *Bacillus clausii* poliantibiotico resistente	Sospensione orale, granulato e capsule
Sigma Tau Industrie Farmaceutiche Riunite	EPTAVIS	*S thermophilus* 48 x10^{9} UFC *B brevis*, *B animalis* 22x 10^{9} UFC *L acidophilus* 460 x 10^{6} UFC *L plantarum* 50 x10^{6} UFC *L paracasei* 50 x10^{6} UFC *L bulgaricus* 70x10^{6} UFC *E faecium* 7x10^{6} UFC	capsule e granulato per sospensione orale
Bruschettini	LACTEOL	*L. fermentum, L. delbrueckii* 5x10^{9} UFC	capsule e granulato per sospensione orale
Li Pharma	LILACTO	*L. plantarum* 10^{8} UFC	capsule molli vaginali
Akkaeda Pharma	MORELAC	*L acidophilus* 10^{7} UFC *L delbrueckii* 5 10^{6} UFC *S thermophilus* 4 x 10^{9} UFC	polvere per sospensione orale
AlfaSigma	YOVIS	*S thermophilus* 204x 10^{9} UFC *B breve, B infantis, B longum* 93x10^{9} UFC *L acidophilus* 2x10^{9} UFC *L plantarum* 220x10^{6} UFC *L casei* minimo 220x10^{6} UFC *L bulgaricus* 300x10^{6} UFC *S faecium* 30x10^{6} UFC	capsule e granulato per sospensione orale

REGULATORY ASPECTS

FOOD

efsa
European Food Safety Authority

L'EFSA, nella valutazione dei claims da autorizzare ai sensi del Regolamento (CE) 1924/2006, sostiene che **"incrementare il numero di un qualsiasi gruppo di batteri" come "aumentare i livelli di microflora benefica" non siano in sé effetti benefici sulla salute"**, e inoltre, che affermazioni come "sostenere una microflora intestinale equilibrata" o "influire beneficamente sulla microflora intestinale" potrebbero essere ritenute benefiche per la salute "in caso di una concomitante diminuzione dei microrganismi potenzialmente patogeni"

EFSA Journal 2009; 7(9) 1232

NO APPROVED HEALTH CLAIM FOR PROBIOTICS

>300 requests
>200 probiotic strains or combinations
>60 beneficial effects claimed

Any claim that imply that contained bacteria are beneficial for health is NOT PERMITTED in EU

FOOD

Ministero della Salute

DIREZIONE GENERALE PER L'IGIENE E LA SICUREZZA DEGLI ALIMENTI E LA NUTRIZIONE - UFFICIO 4

LINEE GUIDA SU PROBIOTICI E PREBIOTICI

Revisione marzo 2018

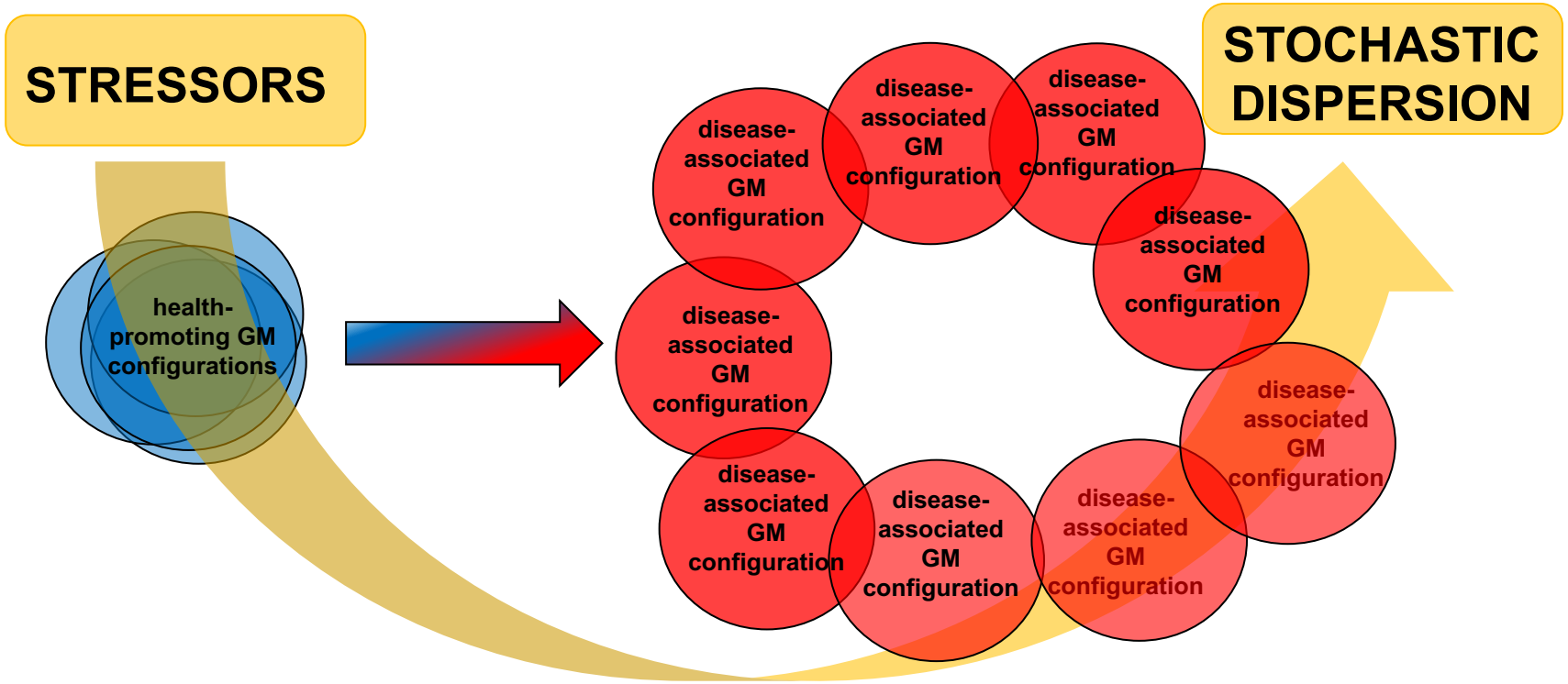
…. prodotti conformi alle presenti linee guida per il loro contenuto di probiotici o prebiotici, **risultando plausibilmente in grado di favorire l'equilibrio della flora batterica, possono indicare in etichetta tale effetto fisiologico ed impiegare termini che lo sottendono come "probiotico"** e "prebiotico".

Identificazione specie e ceppo: introdotto anche sequenziamento genomico (batteri e lieviti)
Quantità dei microrganismi: 10^9 cellule vive / porzione

THE MICROBIAL ECOLOGY OF GM DYSBIOSIS, THE ANNA KARENINA PRINCIPLE

"All happy families are alike; each unhappy family is unhappy in its own way"

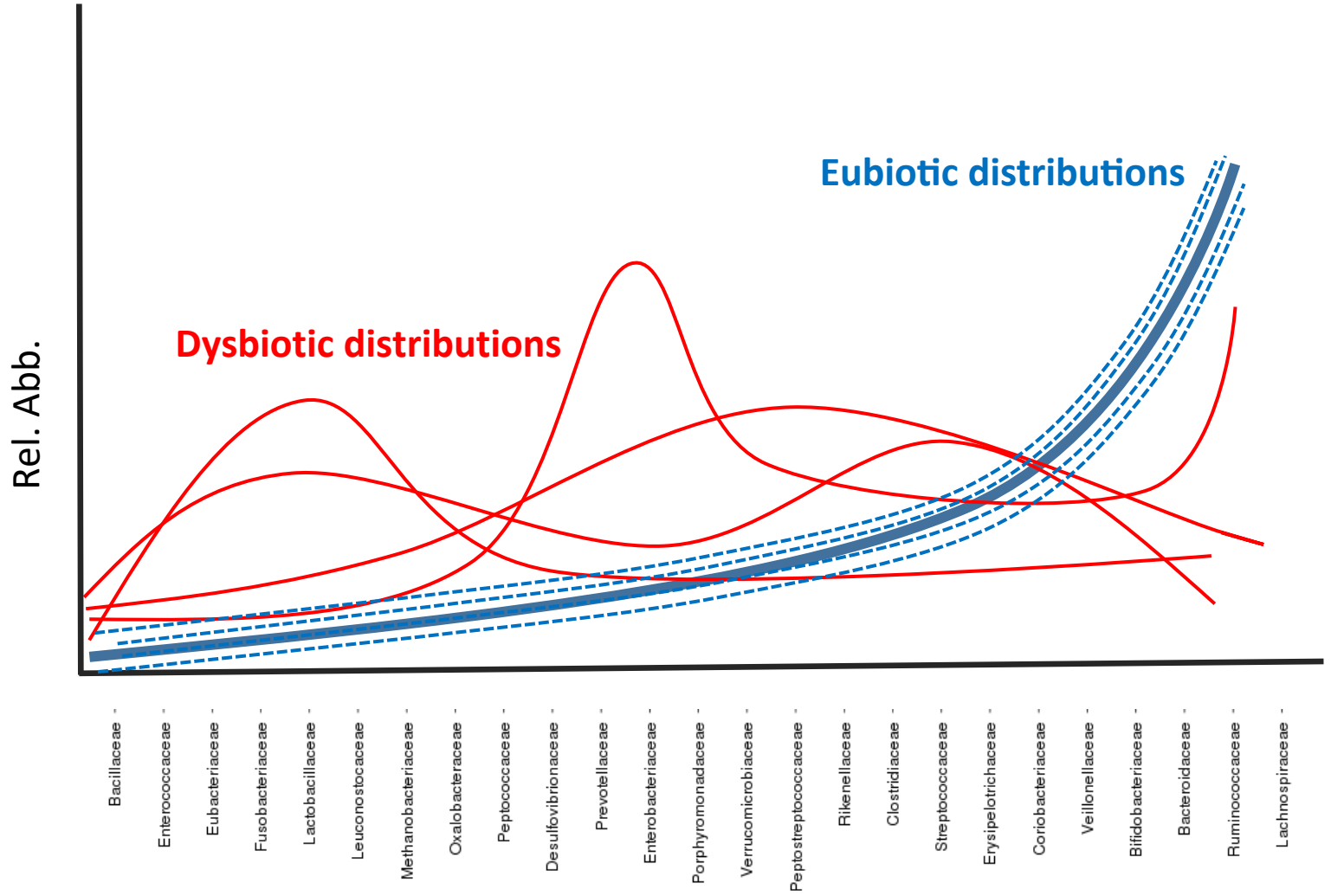
Leo Tolstoy: Anna Karenina (1878)



All microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way

Zaneveld et al., Nat Microbiol 2017

EUBIOTIC AND DYSBIOTIC DISTRIBUTIONS OF THE MAJOR GM FAMILIES



COMMON TRAITS OF DYSBIOSIS

- Reduction of SCFA producing bacteria (butyrate producers such as *Faecalibacterium, Roseburia, Lachnospiraceae, Eubacterium*)
- Increased mucus degradation potential by abnormal mucin degraders that displace *Akkermansia*
- Reduced hydrogen and methane production combined with increased hydrogen sulphide production. H_2S is toxic for the epithelium
- Increase in abundance of bacteria with LPS endotoxins (Proteobacteria) that can drive inflammation
- Increased potential to manage oxidative stress, i.e. microbes became able to proliferate in close vicinity to the epithelium

Le Chatellier et al., Nature 2013

NOVEL CANDIDATED FOR NEW PROBIOTICS

Faecalibacterium prausnitzii
Roseburia spp.
Eubacterium hallii
Akkermansia muciniphila

Demonstration of safety and efficacy strain-specific

Faecalibacterium prausnitzii
No regulatory approval as a probiotic.
Future probiotic uses are likely to occur when the safety of the species has been demonstrated to satisfy regulatory authority

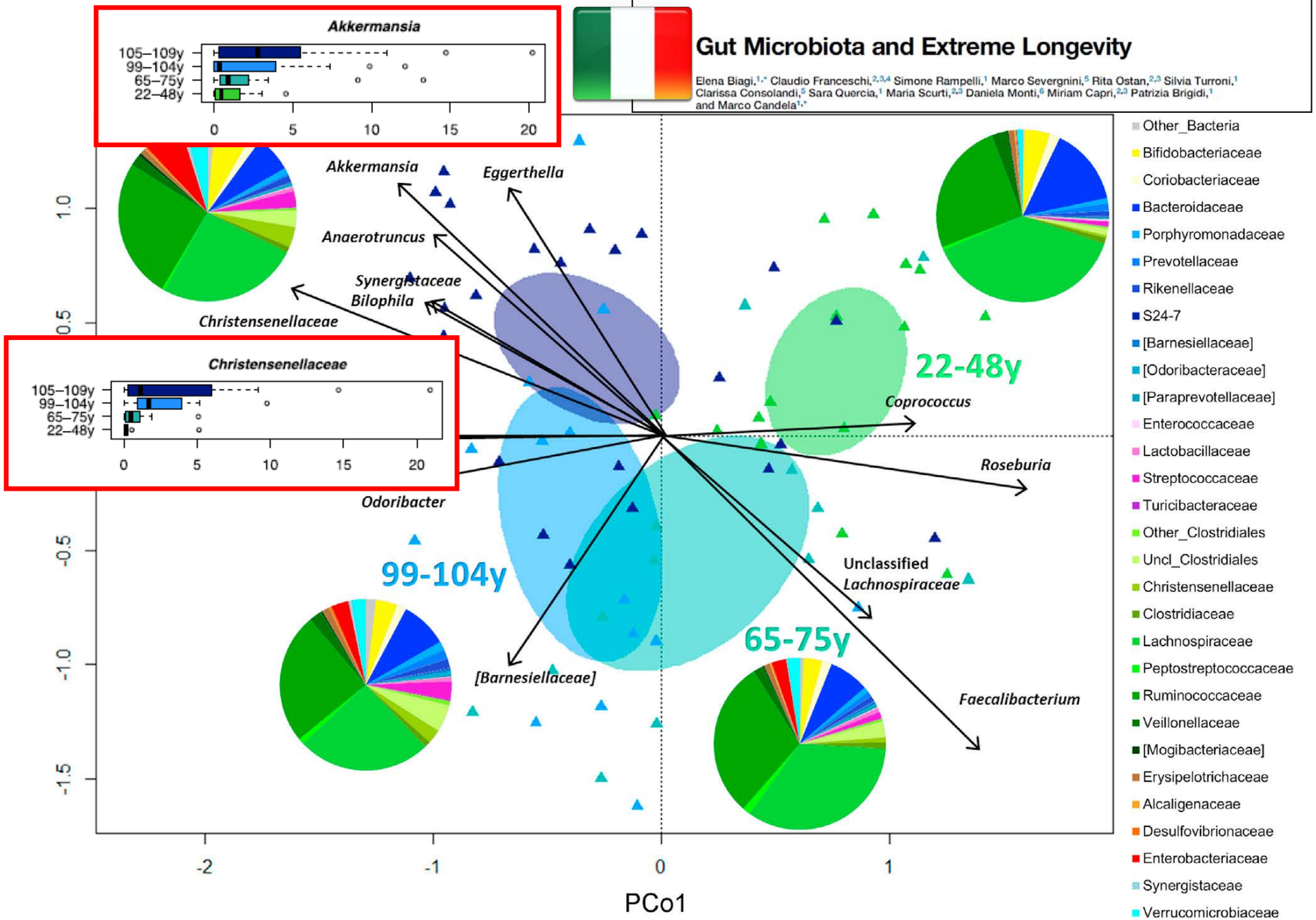
SUGGESTED INFORMATION REQUIREMENTS FOR NOVEL PROBIOTICS

- ✓ ***Complete genome announcement and annotation***. Functional annotation would help in predicting function.
- ✓ ***Antibiotic resistance profile*** and conjugation studies to study transferability of antibiotic resistance.
- ✓ ***Selection of proper in vivo model***: mouse and rat models do not provide the 'actual' gastrointestinal conditions of humans. Preliminary testing could be essential for newly characterised strains or species.
- ✓ ***Toxicological studies*** should be scientifically assessed that the species or strain claimed for its probiotic properties does not produce any toxins.
- ✓ ***Target population*** should be clearly defined (a probiotic found to be effective in one population may have some adverse effect in another due to varied susceptibility to particular microbes).

CellPress
Current Biology
Report

Gut Microbiota and Extreme Longevity

Elena Biagi,[1,*] Claudio Franceschi,[2,3,4] Simone Rampelli,[1] Marco Severgnini,[5] Rita Ostan,[2,3] Silvia Turroni,[1] Clarissa Consolandi,[5] Sara Quercia,[1] Maria Scurti,[2,3] Daniela Monti,[6] Miriam Capri,[2,3] Patrizia Brigidi,[1] and Marco Candela[1,*]



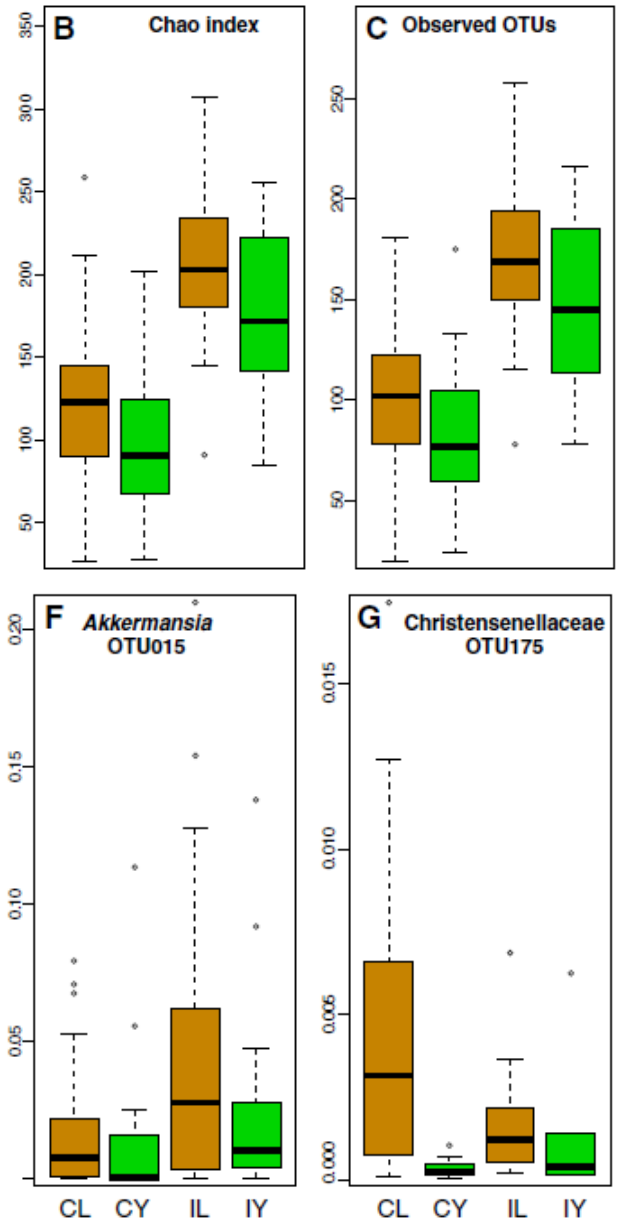
CellPress

Correspondence

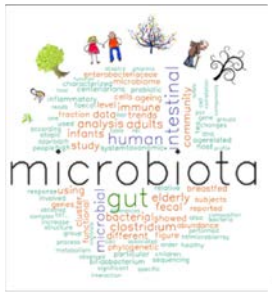
Gut microbiota signatures of longevity

Fanli Kong[1,2,4], Yutong Hua[1,4], Bo Zeng[1], Ruihong Ning[1], Ying Li[1,3,5,*], and Jiangchao Zhao[2,5,6,*]

Comparison between Italian (I) and Chinese (C) **centenarians** and **young adults**



Akkermansia and *Christensenellaceae* can represent a signature of adaptation to the changes associated with the long living, regardless of lifestyle and dietary habits.




Patrizia Brigidi
Full Professor


Silvia Turroni
Assistant Professor


Monica Barone
PhD student


Federica D'Amico
PhD student


Margherita Musella
PhD student


Simone Rampelli, PhD


Elena Biagi, PhD


Giorgia Palladino
PhD student


Matteo Soverini
PhD student


Marco Candela
Associate Professor

Microbial Ecology of Health UNIT

Dept. Pharmacy and Biotechnology
University of Bologna, Italy

THANK YOU FOR YOUR ATTENTION !!!

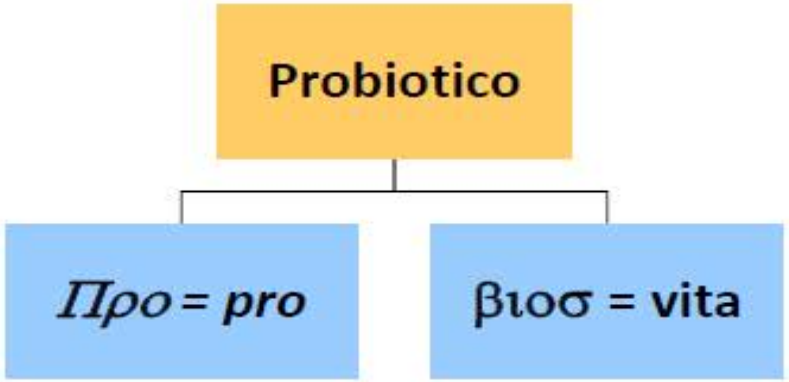
European Commission

Circles

Controlling Microbiomes Circulations for Better Food Systems
H2020-SFS-2018-2020

1

PROBIOTICS DEFINITION



live microorganisms which when administered in adequate amounts confer a health benefit on the host

WHO (FAO/WHO), 2001

live microorganisms that, when administered in adequate amounts, confer a health benefit on the host

International Scientific Association for Probiotics and Prebiotics (ISAPP), 2014

Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

COMMON PROBIOTIC TRAITS

GENOME SEQUENCING

- Adhesion to the host
- Efflux systems to survive gastric environment
- Hydrolases to confer bile-salt tolerance
- Metabolism optimized for conversion of carbohydrates to lactic acid and, in some cases, a mixture of other acids
- Rapid acidification and low-to-moderate growth yield

3

INGESTED BACTERIA – MEDIATED MICROBIOME

Ingested microbial cells:10^9

Firmicutes Bacteroidetes Proteobacteria Actinobacteria Other

	Taxonomy (phylum level)	Resident bacteria (number/ml or g)	Transit time[a]	Relative abundance of ingested bacteria compared to resident bacteria[b]
Stomach[c]		10^2–10^4	15 min–3 h	100 to 10 000-fold
Small intestine (ileum)[d]		10^6–10^8	2–5 h	0.01 to 1-fold
Colon (feces)[e]		10^{10}–10^{11}	12–24 h	0.0001 to 0.00001-fold

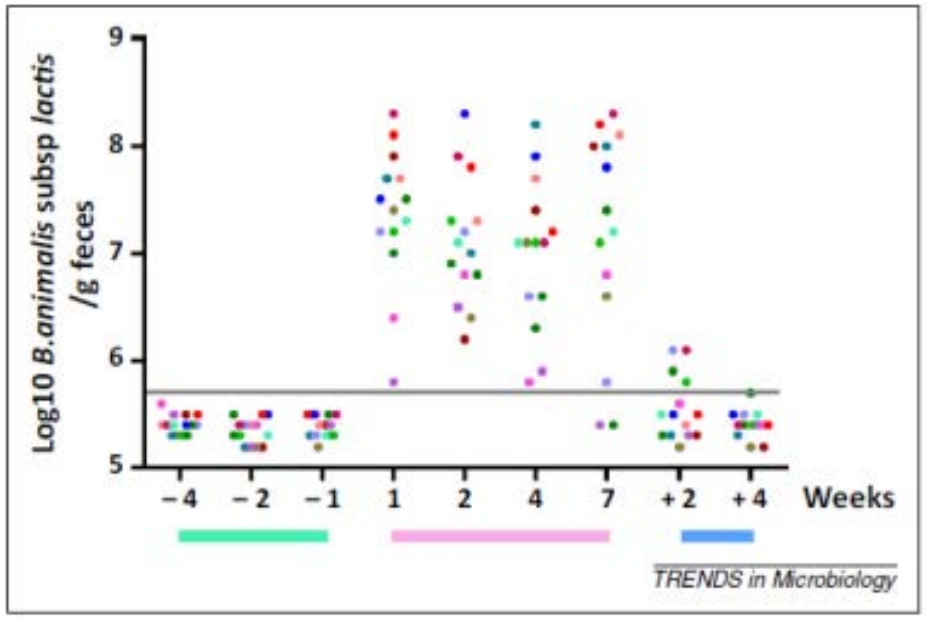
Derrien and Vlieg, 2015, Trends Microbiol

FATE OF INGESTED STRAIN

«PERSISTENCE» = fecal quantification of the ingested strain, reflecting the extent of cell death and subsequent replication of surviving cells

Strain dependent

High inter-individual variability (strains are rarely detected after 1 w)



Preconsumption period

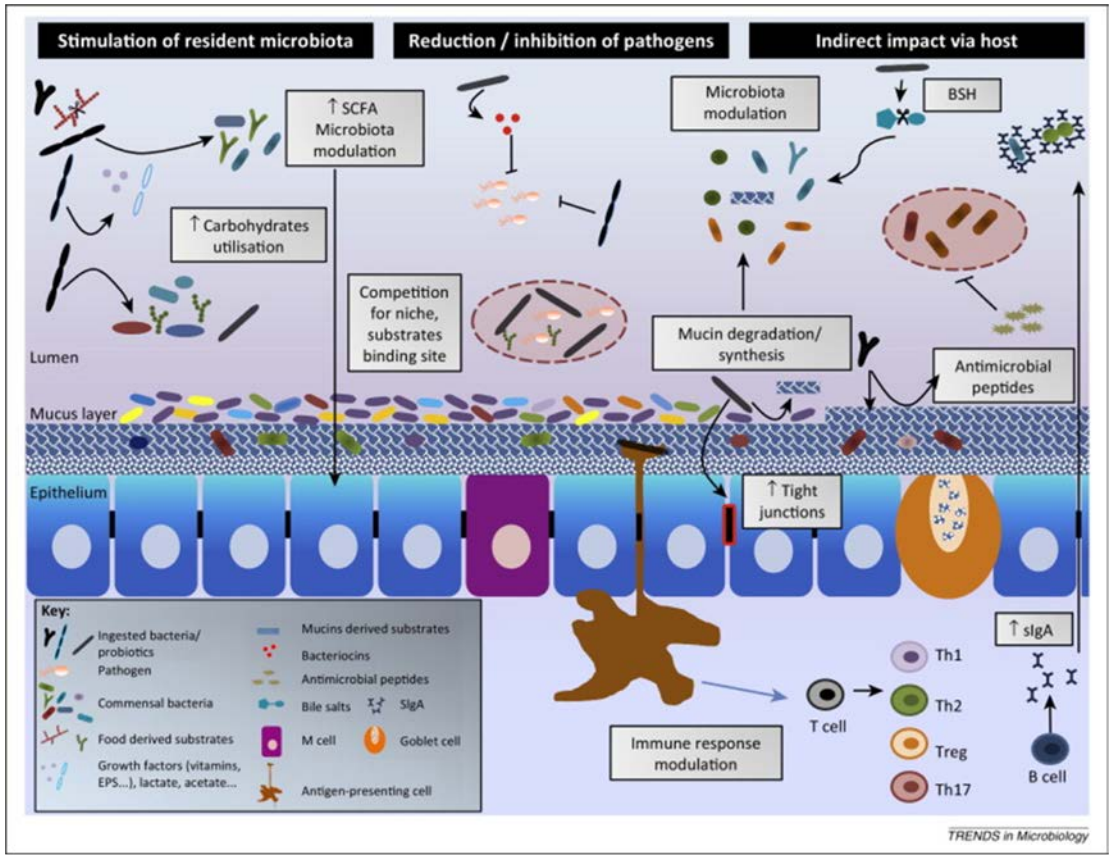
Consumption period

Postconsumption period

Derrien and Vlieg, 2015, Trends Microbiol

5

TRANSIENT INTEGRATION IN THE GUT MICROBIOTA ("TRANSIENT MICROBIOTA")

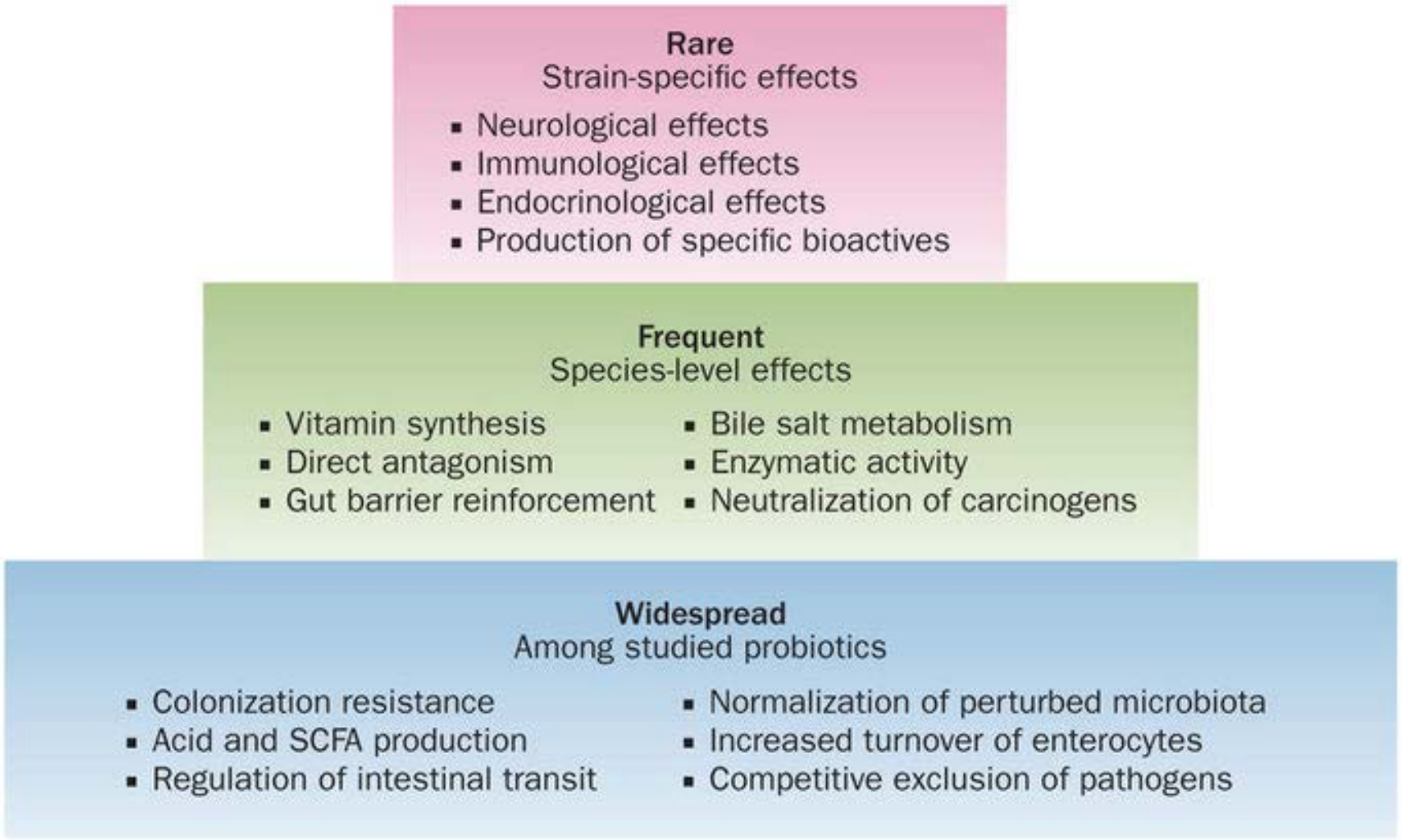


TRANSIENT MICROBIOME CAN IMPACT ON COMPOSITION AND ACTIVITY OF THE RESIDENT COMMUNITY:

- ✓ ***Stimulation of resident microbiota by trophic interaction*** *(metabolites, growth factors, carbohydrate metabolism, mucin degradation)*
- ✓ ***Reduction/inhibition of pathogens*** *through alteration of the microbial fitness (**pH decrease, niche competition, EPS and bacteriocins**)*
- ✓ *Indirect impact via host through **changes in the gut environment** (mucin production, increase of IgA and defensins)*

Derrien and Vlieg, 2015, Trends Microbiol

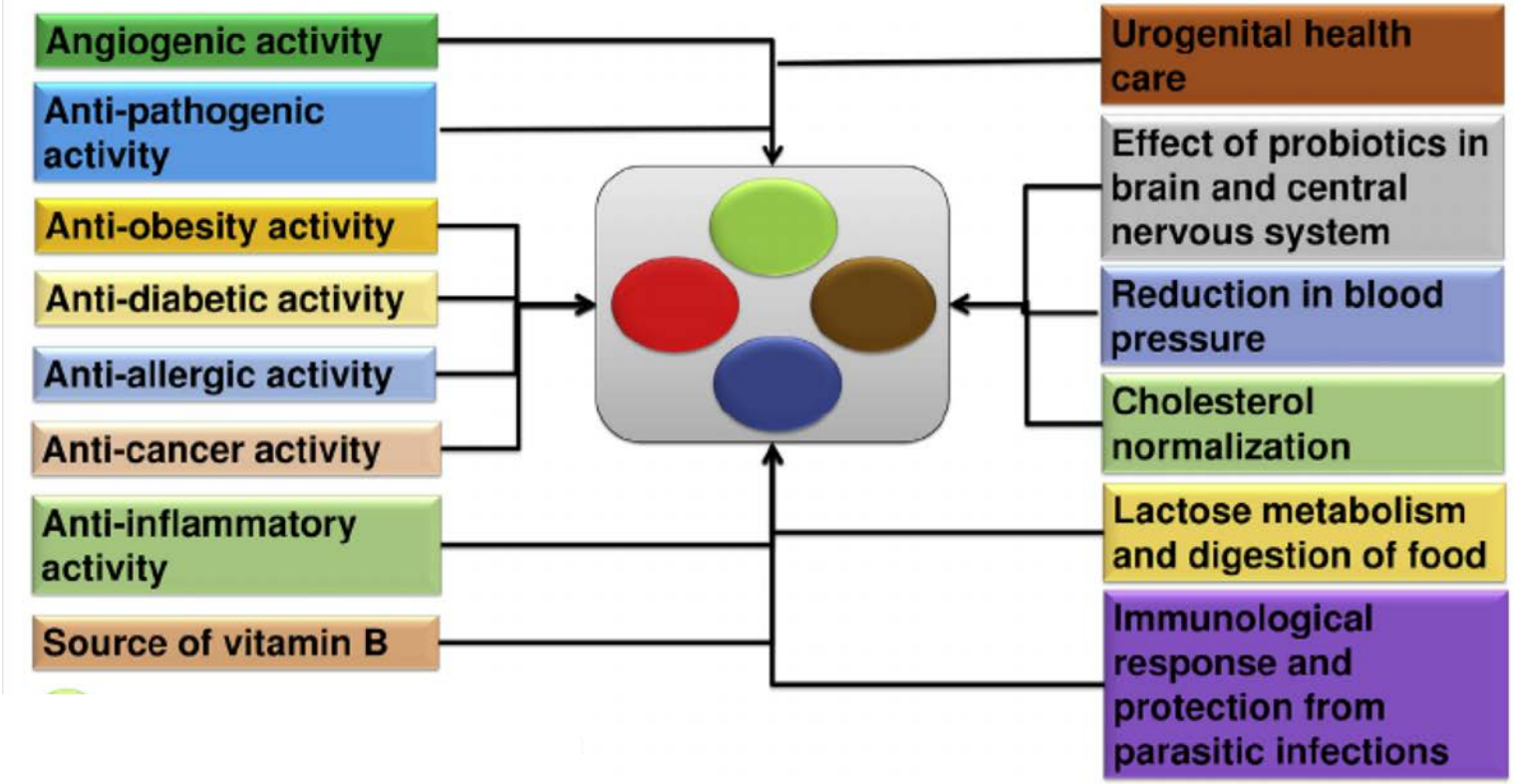
POSSIBLE DISTRIBUTION OF MECHANISMS AMONG PROBIOTICS



Hill et al, Nature Reviews Gastroenterology & Hepatology, 2014

APPLICATIONS OF PROBIOTICS AND THEIR MODE OF ACTION

(strain specific)



Kerry GR, et al., J Food and Drug Anal, 2018

MEASURE OF THE PROBIOTIC EFFECTS: HERE COMES THE PROBLEM

STUDIES ON HUMAN

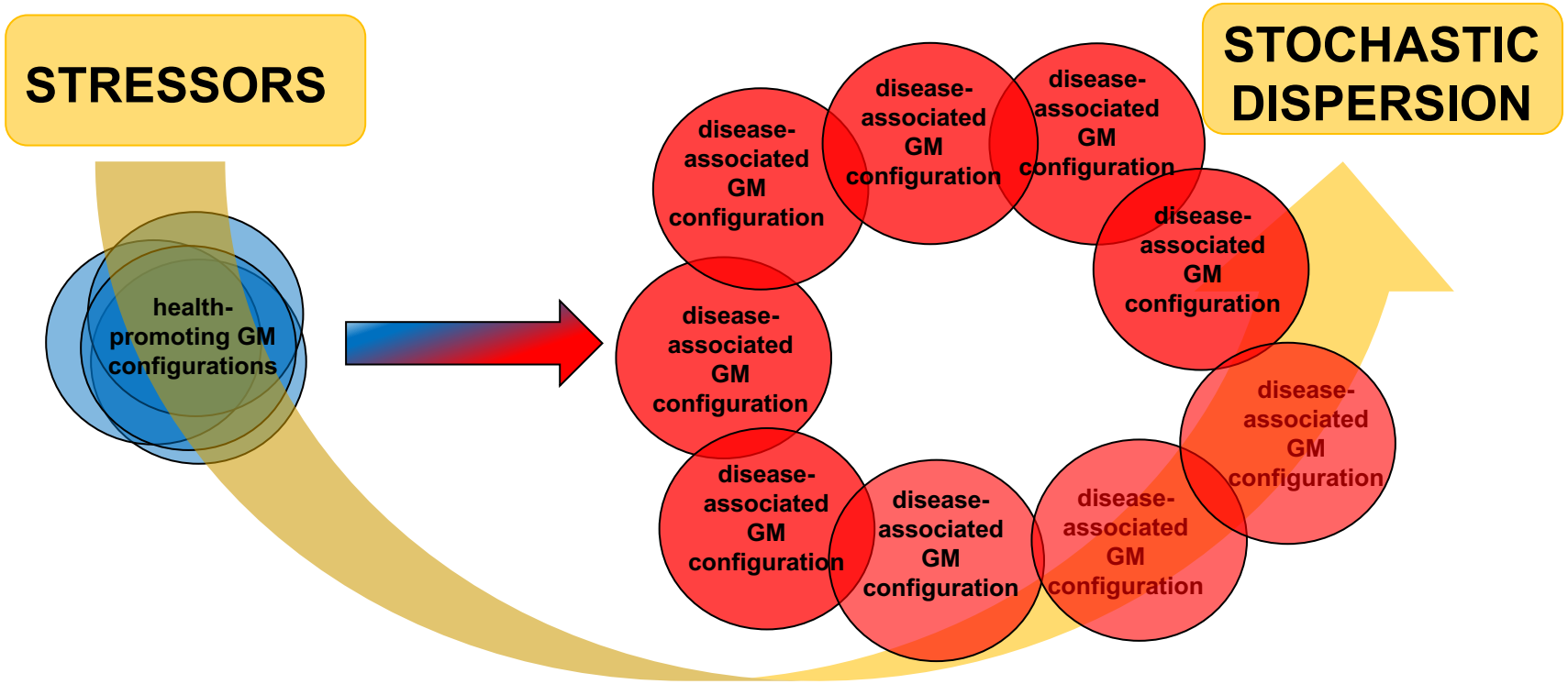
- **Heterogeneity of chosen strain (or mix of strains), duration of the intervention, dosage**
- **Heterogeneity of matrix (dairy food, capsule, powder…)**
- **Study design (comparison with placebo or baseline)**
- **Chosen population (adults, children, elderly; health, disease)**
- **Evaluation of the effects on health (long term, short term; therapeutic, preventive; …)**
- **Evaluation of the effect on the resident microbiota (different techniques)**

Derrien and Vlieg, 2015, Trends Microbiol

THE MICROBIAL ECOLOGY OF GM DYSBIOSIS, THE ANNA KARENINA PRINCIPLE

"All happy families are alike; each unhappy family is unhappy in its own way"

Leo Tolstoy: Anna Karenina (1878)



All microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way

Zaneveld et al., Nat Microbiol 2017

SUGGESTED INFORMATION REQUIREMENTS FOR NOVEL PROBIOTICS

- ✓ ***Complete genome announcement and annotation***. Functional annotation would help in predicting function.
- ✓ ***Antibiotic resistance profile*** and conjugation studies to study transferability of antibiotic resistance.
- ✓ ***Selection of proper in vivo model***: mouse and rat models do not provide the 'actual' gastrointestinal conditions of humans. Preliminary testing could be essential for newly characterised strains or species.
- ✓ ***Toxicological studies*** should be scientifically assessed that the species or strain claimed for its probiotic properties does not produce any toxins.
- ✓ ***Target population*** should be clearly defined (a probiotic found to be effective in one population may have some adverse effect in another due to varied susceptibility to particular microbes).