14° CONGRESSO NAZIONALE SINut

SINut Società Italiana di Nutraceutica

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Bologna

DAL MICROBIOTA AL PROBIOTICO: UN PROCESSO COMPLESSO

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La sottoscritta Silvia Turroni
ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

dichiara

che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario

OUTLINE

- Probiotics: consensus statement, benefits and underlying mechanisms
- Caveats in the probiotics field and proposed strategies to overcome them
- … Towards next-generation probiotics and postbiotics

CONSENSUS PANEL RECOMMENDATIONS FOR THE SCOPE OF PROBIOTICS

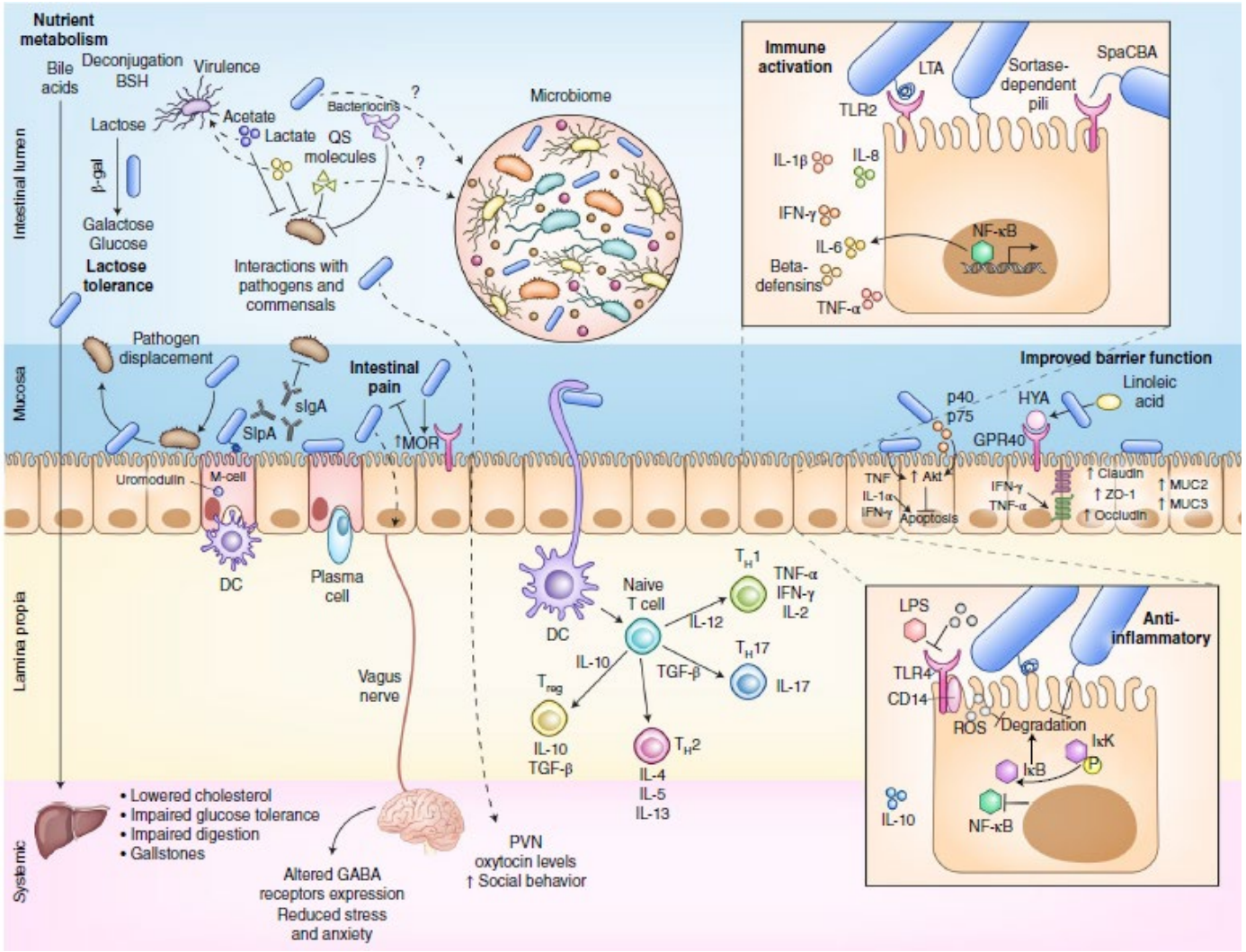
Box 1 | Consensus panel recommendations for the scope of probiotics

- Retain the FAO/WHO definition[1] for probiotics, with a minor grammatical correction as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"; inconsistences between the Expert Consultation[1] and the FAO/WHO Guidelines[2] were clarified
- Include in the framework for definition of probiotics microbial species that have been shown in properly controlled studies to confer benefits to health
- Any specific claim beyond "contains probiotics" must be further substantiated
- Keep live cultures, traditionally associated with fermented foods and for which there is no evidence of a health benefit, outside the probiotic framework
- Keep undefined, faecal microbiota transplants outside the probiotic framework
- New commensals and consortia comprising defined strains from human samples, with adequate evidence of safety and efficacy, are 'probiotics'

Abbreviation: FAO, Food and Agriculture Organization of the United Nations.

«LIVE MICROORGANISMS THAT, WHEN ADMINISTERED IN ADEQUATE AMOUNTS, CONFER A HEALTH BENEFIT ON THE HOST»

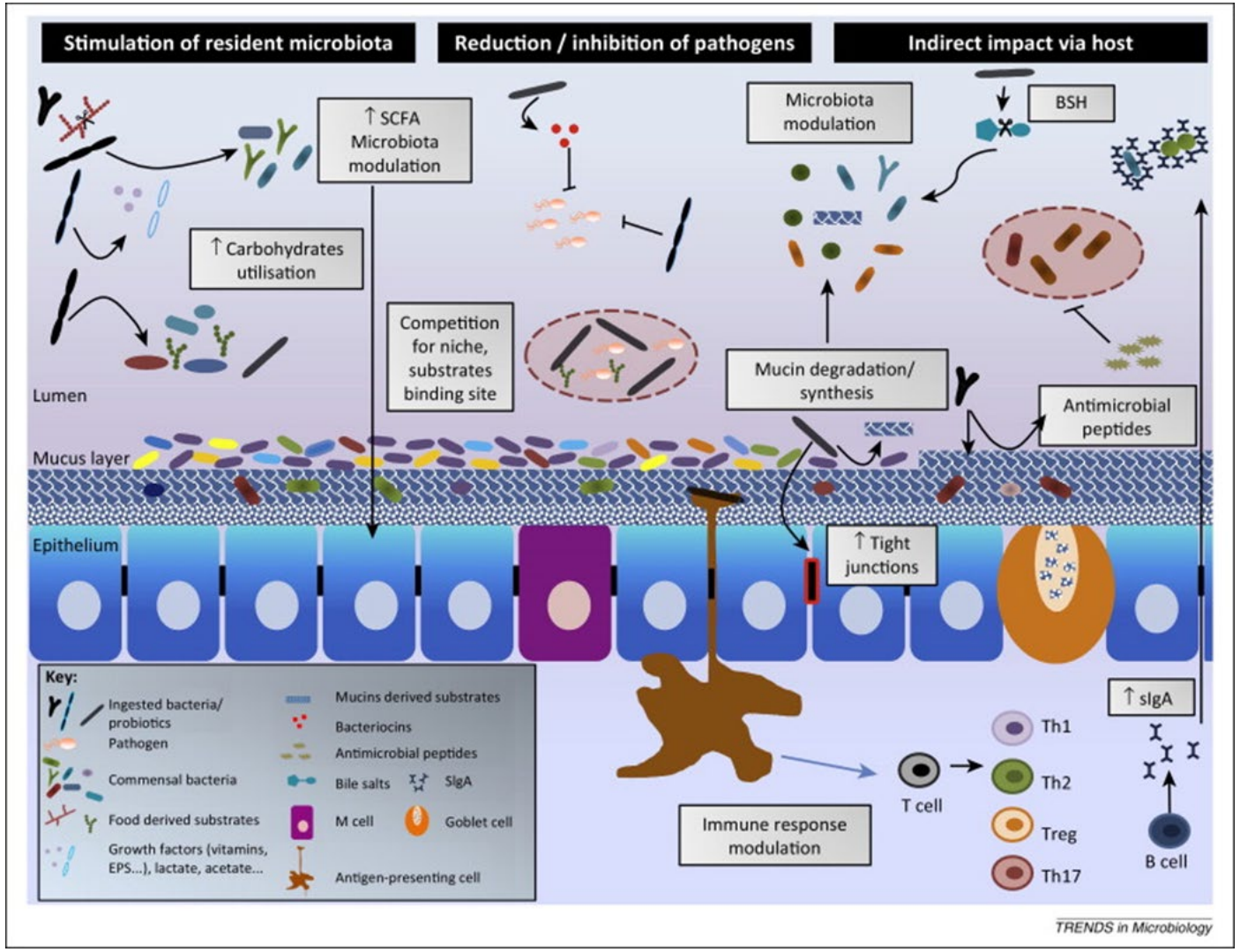
Hill *et al.*, Nat Rev Gastroenterol Hepatol. 2014



Suez *et al.*, Nat Med. 2019

MECHANISTIC INTERACTIONS BETWEEN PROBIOTICS AND THE HOST AND ITS MICROBIOME

- ✓ Metabolism of nutrients
- ✓ Direct and indirect pathogen antagonism
- ✓ Improved barrier function
- ✓ Alteration of the microbiome
- ✓ Change of signaling to the nervous system
- ✓ Immunomodulation
- ✓ Reduction of visceral pain
- ✓ …

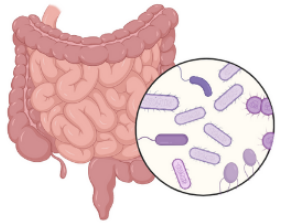


Derrien *et al.*, Trends Microbiol. 2015

IMPACT ON THE GUT MICROBIOME

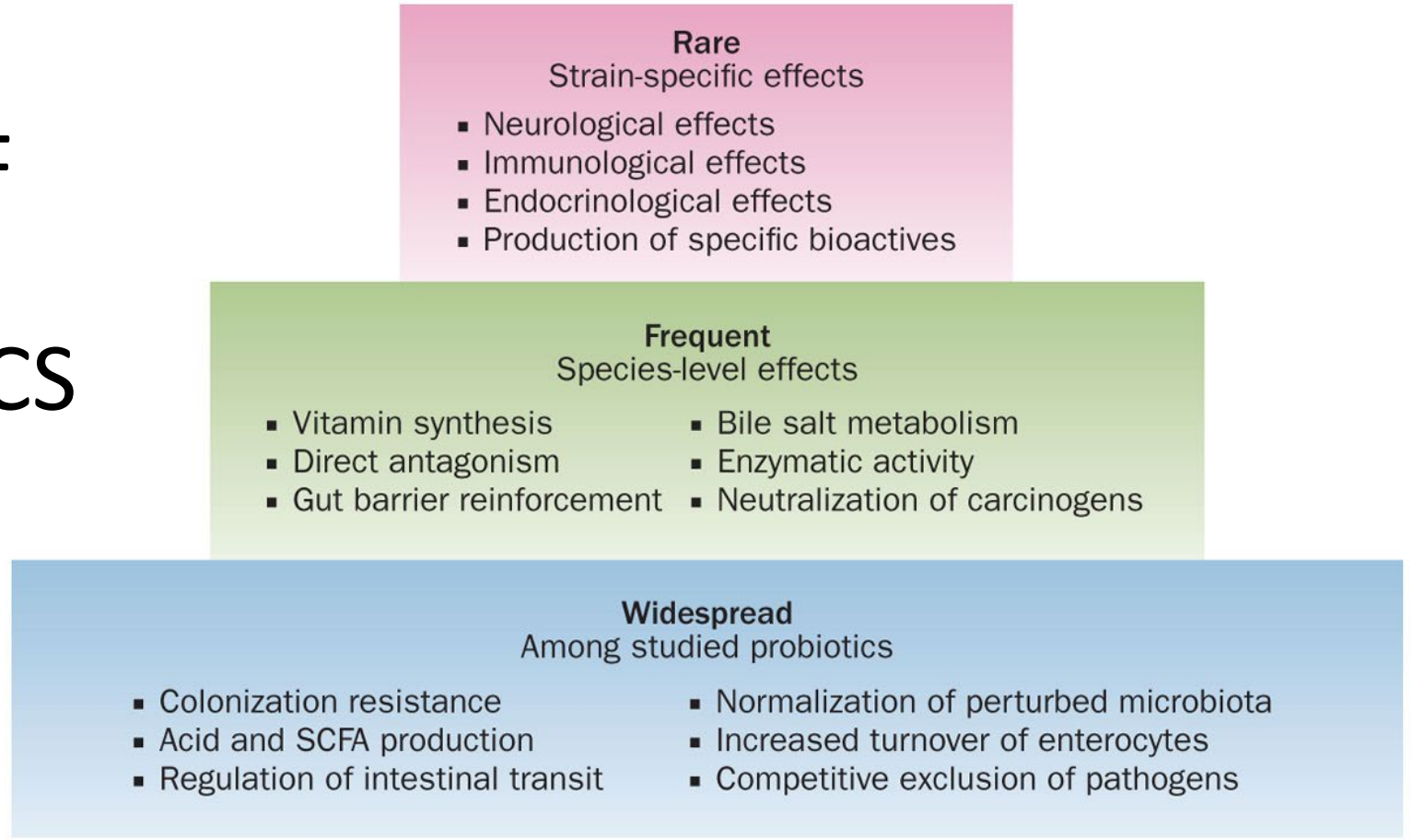
- ✓ Stimulation of resident microbiota by trophic interactions (metabolites, growth factors, carbohydrate metabolism, mucin degradation)
- ✓ Reduction/inhibition of pathogens through alteration of the microbial fitness (pH decrease, niche competition, EPS and bacteriocins)
- ✓ Indirect impact via host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

EXTRA-GASTROINTESTINAL EFFECTS OF PROBIOTICS



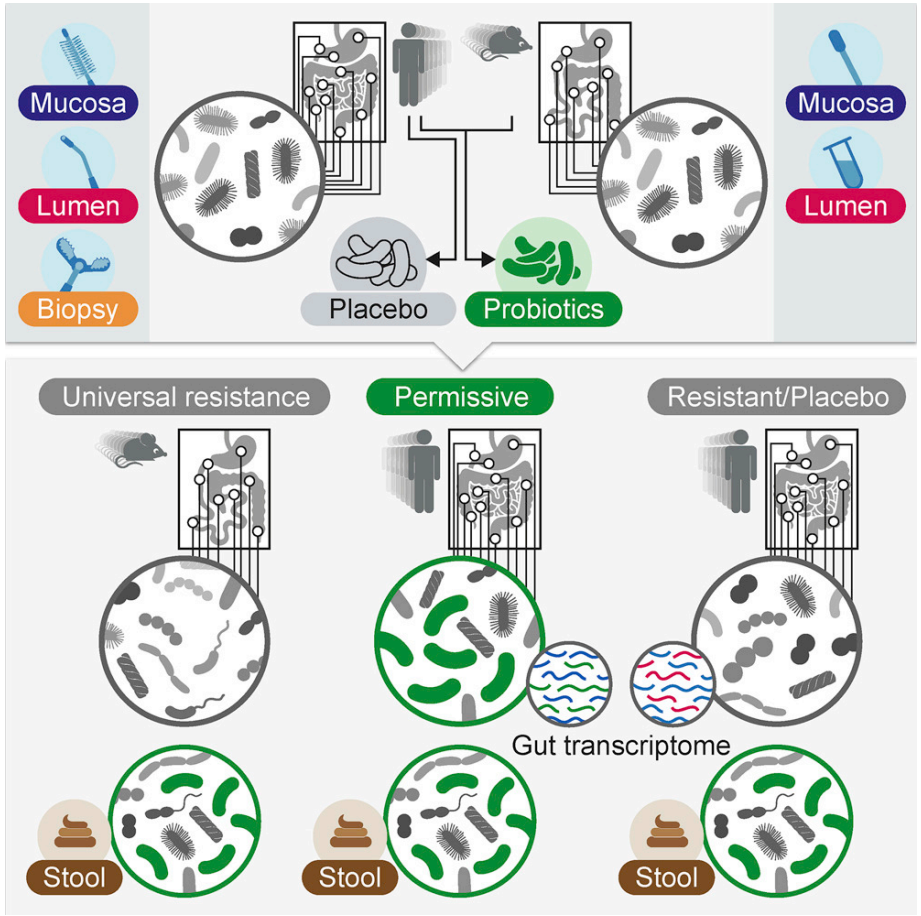
Roggiani *et al.*, 2024

POSSIBLE DISTRIBUTION OF MECHANISMS AMONG PROBIOTICS



Hill *et al.*, Nat Rev Gastroenterol Hepatol. 2014

ONE SIZE DOES NOT FIT ALL



Zmora *et al.*, Cell. 2018

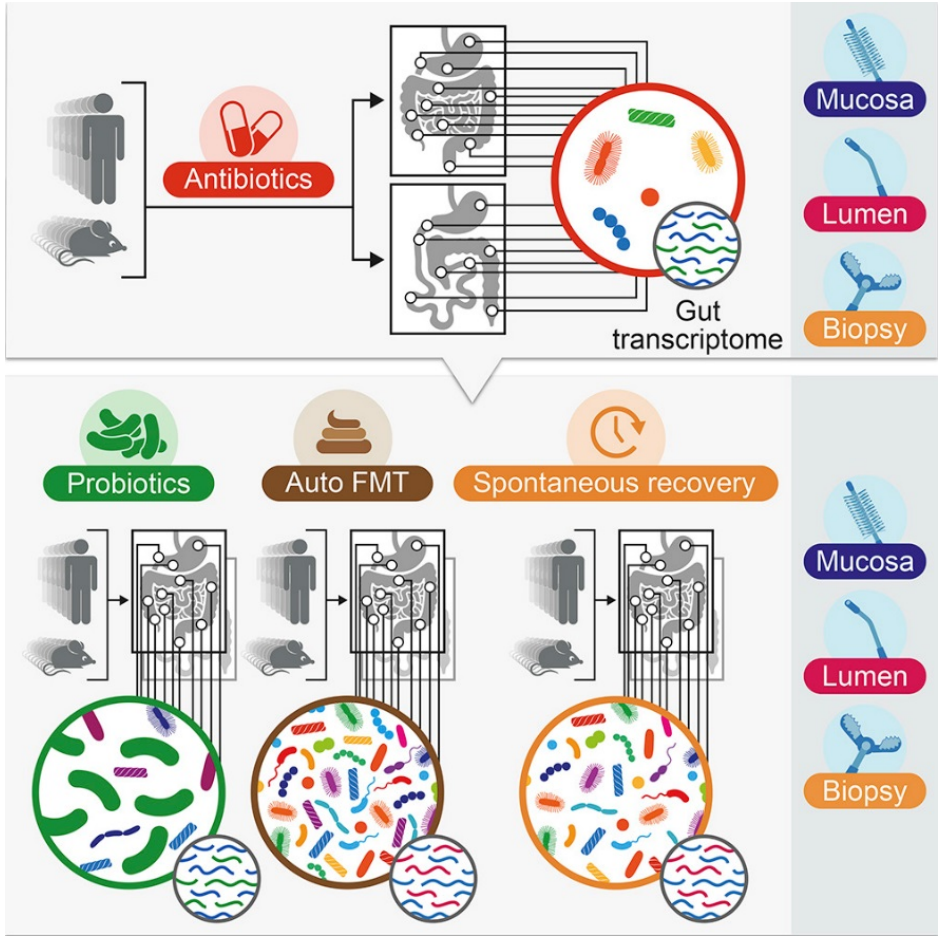
Humans feature a **person-specific gut mucosal colonization resistance to probiotics**

Probiotics colonization is predictable by pre-treatment microbiome and host features

ONE SIZE DOES NOT FIT ALL

Probiotics may perturb rather than aid in microbiota recovery back to baseline after antibiotic treatment in humans (unlike aFMT)

If not tailored, probiotic interventions may be not risk free



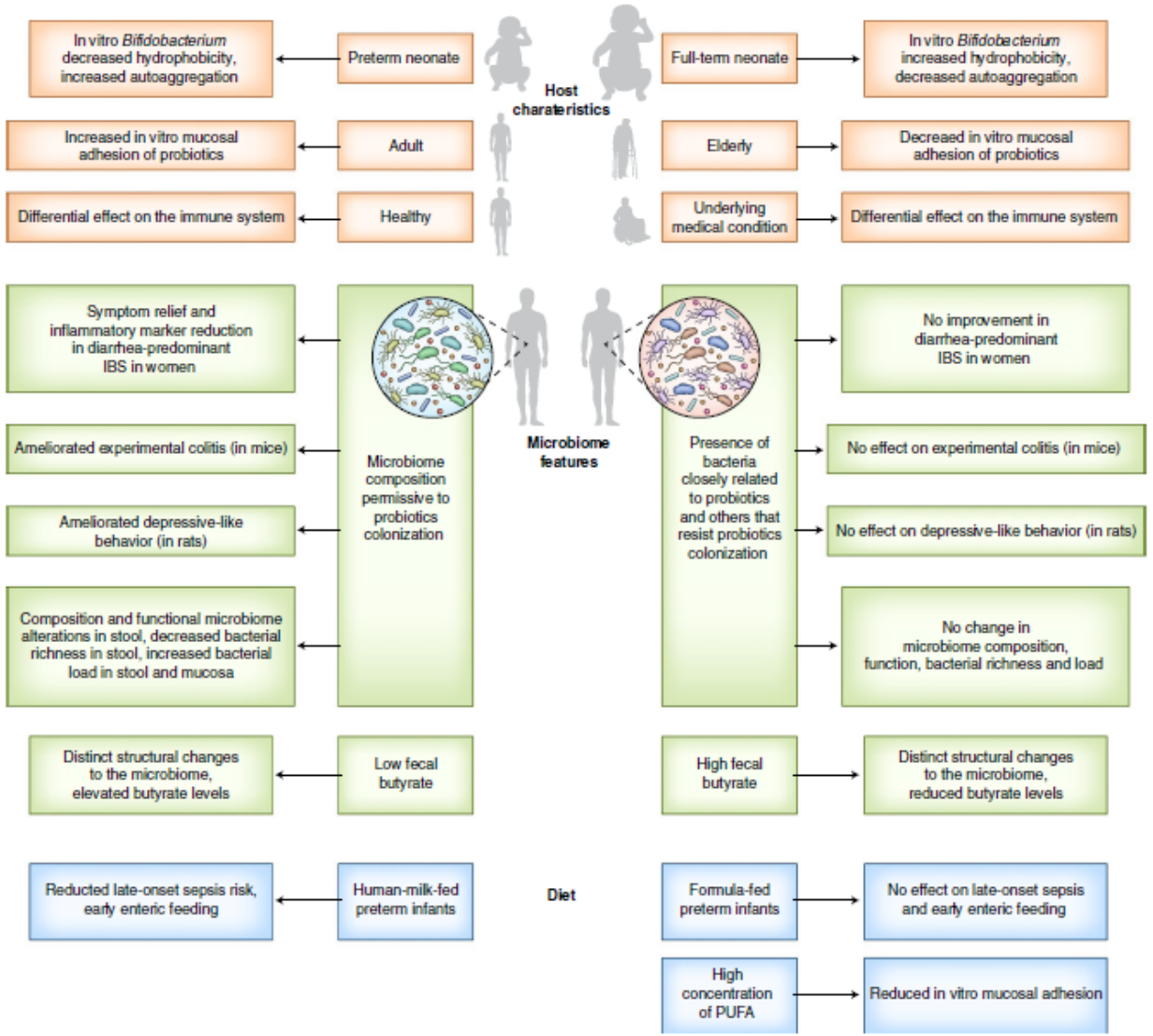
Suez *et al.*, Cell. 2018

CAVEATS IN THE PROBIOTICS FIELD AND PROPOSED STRATEGIES TO OVERCOME THEM

Table 1 | Caveats in the probiotics field and proposed strategies to overcome them

Limitation	Current state	What can be done
Conception	Probiotics often regarded as a homogenous entity	Strain-level resolution of clinical and mechanistic studies
		Avoid bundling of strains in analyses
Spectrum	Strain selection confined to few genera	Novel candidate microorganisms with suggested health benefits from recent microbiome research
Research approach	Trial and error–based	Mechanism-based
Research methodology	Sample size inadequate at times	Sample size based on power analysis
	Endpoints indirect, irrelevant and/or poorly or subjectively defined	Highly valid and reliable endpoints
		Account for placebo effect
	Adverse events under-reported	Report adverse events and side effects
Sampled material	Effect evaluated remotely from target site (stool)	Effect evaluated in situ through endoscopic sampling
Reliance on models	In vitro models lack probiotics–microbiome and probiotics–host mucosal interactions	Human trials as the mainstay of probiotic research; in vivo and In vitro experimentation used to validate human trials and further explore mechanisms of action
	In vivo models may not be compatible with human probiotics	
Stratification and personalization	One-size-fits-all therapy	Precision therapy based on host and microbiome characteristics, as well as diet
Safety	Insufficient reporting of safety outcomes, especially in the long term	Long-term safety, especially for critically ill and immune-compromised individuals, as an obligatory quality-control measure
Motivation	Driven by commercial interests	Driven by medical interests
	Regulated as dietary supplements, so proof of efficacy not mandatory	Regulated as drugs, so proof of efficacy under scrutiny by medical authorities

Suez *et al.*, Nat Med. 2019



Suez *et al.*, Nat Med. 2019

STRATIFICATION AND PERSONALIZATION

Distinct initial conditions in the host and their microbiome and varying environmental exposures can result in differing outcomes in different individuals who are supplemented with the same probiotic preparation.

- ✓ **Underlying medical conditions** are known to modify the effects of probiotics on immune cells
- ✓ **Features of the indigenous microbiome** (permissive/resistant to colonization) can account for different impacts of probiotics on the host
- ✓ **Presupplementation butyrate levels** are associated with a differential probiotic effect on the microbiome and butyrate production or metabolism
- ✓ **Diet** may affect probiotic properties and clinical outcome

NEXT-GENERATION PROBIOTICS: THE SPECTRUM FROM PROBIOTICS TO LIVE BIOTHERAPEUTICS

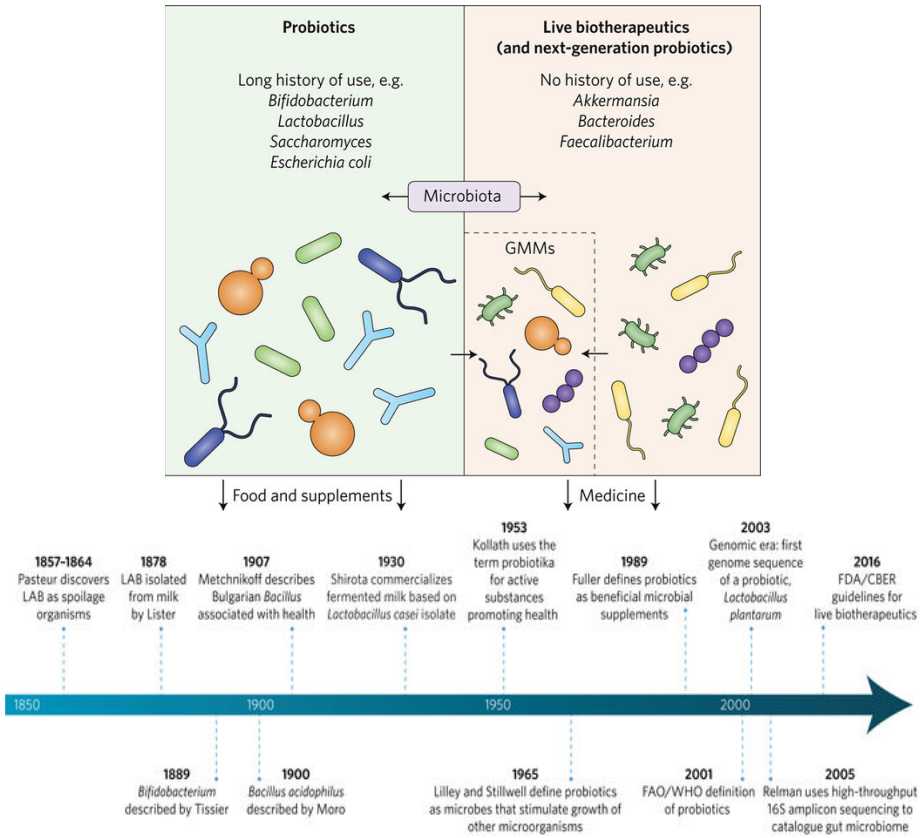
FDA definition of LBP:

A biological product that:

(1) contains live organisms, such as bacteria

(2) is applicable to the prevention, treatment, or cure of a disease or condition of human being

(3) is not a vaccine



O'Toole *et al.*, Nat Microbiol. 2017

NEXT-GENERATION PROBIOTICS: THE SPECTRUM FROM PROBIOTICS TO LIVE BIOTHERAPEUTICS

Dominant gut microbiota members in adults with a key functional role

Faecalibacterium prausnitzii, butyrate producer with anti-inflammatory and immunomodulation properties, for IBD

Akkermansia muciniphila, mucolytic component, anti-inflammatory (mucus thickness, antimicrobial peptides, 2-OG, Foxp3 regulatory T cells), promising for obesity treatment

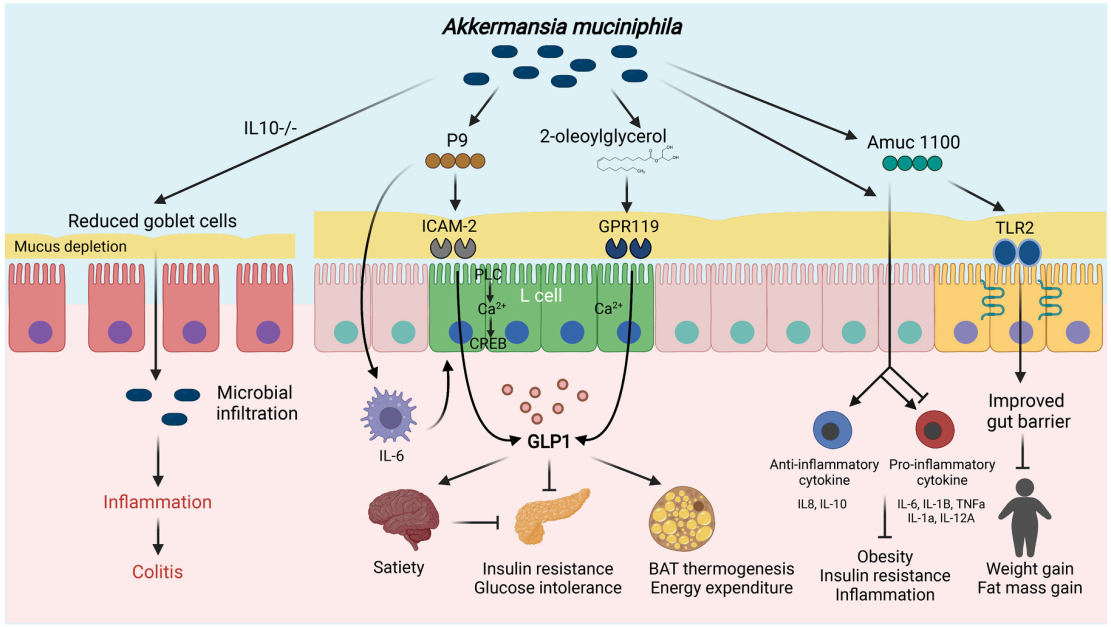
Christensella, anti-inflammatory potential, correlated with leanness, with promising application to support healthy ageing

Bacteroides ovatus & B. xylanisolvens, enhanced cancer immune surveillance

Roseburia&Bacteroides, anti-*Candida* growth and anti-virulence activities (TOR pathway), for antagonistic inter-kingdom application

Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study

Depommier *et al.*, Nat Med. 2019



Si *et al.*, Gut Microbes. 2022

Following a request from the European Commission, **the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on pasteurised *Akkermansia muciniphila* as a novel food (NF) pursuant to Regulation (EU) 2015/2283.** *A. muciniphila* is a well-characterised non-toxin producing, avirulent microorganism that has been reported as part of normal gut microbiota. The NF, pasteurised *A. muciniphila*, is proposed by the applicant to be used as a food supplement at max. 5×10^{10} cells/day by **adults excluding pregnant and lactating women**, and in foods for special medical purposes. The Panel considers that the production process of the NF is sufficiently described and that the information provided on the composition of the NF is sufficient for its characterisation. Taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. Based on literature data, and by applying an uncertainty factor of 200 to the no observed adverse effect level (NOAEL) of a 90-day repeated dose oral toxicity study in rats, **the Panel concludes that the consumption of 3.4×10^{10} cells/day is safe for the target population under the provision that the number of viable cells in the NF is < 10 colony forming units (CFU)/g (*i.e.*, limit of detection).**

EFSA Journal 2021;19(9):6780

A. muciniphila is currently available on the market in pasteurized form for weight management and glycemic control

NEXT-GENERATION PROBIOTICS: THE SPECTRUM FROM PROBIOTICS TO LIVE BIOTHERAPEUTICS

An alternative route to developing next-generation probiotics is to take GRAS (generally recognized as safe) organisms or commensals and use them as a delivery vehicle for a bioactive molecule or to introduce new functions

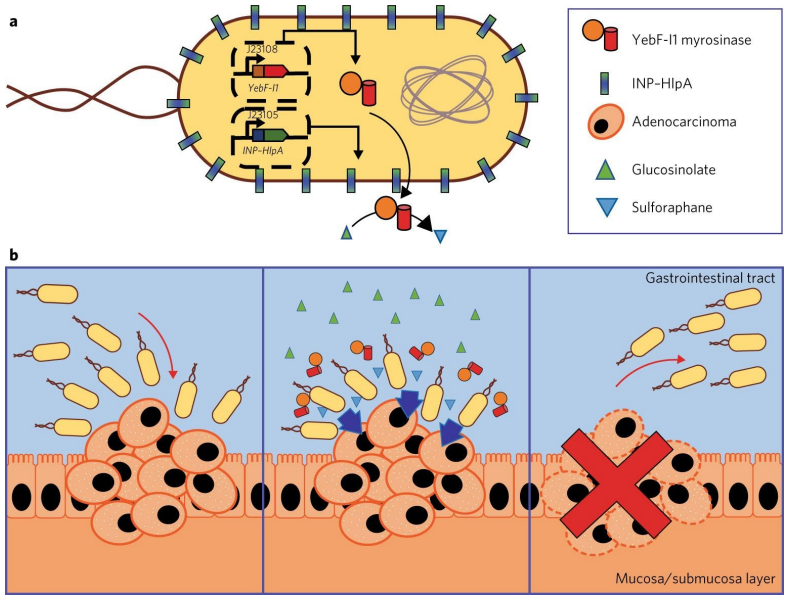
Table 1 | Selected examples of next-generation probiotics.

Organism	Type	Disease target	Level of evidence	Study type	Ref.
Bacteroides xylanisolvens DSM 23694	Natural (human)	Cancer	Medium: safety in humans has been established while levels of TFα-specific IgM have been shown to be elevated in humans	Human	10
Bacteroides ovatus D-6	Natural (human)	Cancer	Low to medium: increases levels of murine TFα-specific IgM and IgG	Preclinical in mice	37
Bacteroides ovatus V975	GMO (originally from human gut samples) expressing KGF-2	Intestinal inflammation	Medium: shows abrogation of symptoms of DSS induced in murine colitis model	Preclinical in mice	25
Bacteroides ovatus V975	GMO expressing TGF-β1	Intestinal inflammation	Medium: shows abrogation of symptoms of DSS induced in murine colitis model	Preclinical in mice	26
Bacteroides dorei D8	Natural (human)	Heart disease	Low: depletion of cholesterol *in vitro*	Preclinical *in vitro*	38
Bacteroides fragilis ZY-312	Natural (human)	Clearance of infectious agents	Low: data only *in vitro*	Preclinical *in vitro*	4
Bacteroides acidifaciens JCM 10556(T)	Natural (mouse)	Clearance of infectious agents	Low to medium: increases IgA levels in the large intestine of gnotobiotic mice	Preclinical in mice	11
Clostridium butyricum MIYAIRI 588	Natural (human)	Multiple targets including cancer, inflammation and infectious agents	Low to medium: evidence gathered for claims in human and animals trials	Human	12–16, 39–51
Faecalibacterium prausnitzii	Natural (human)	Mainly IBD but also asthma, eczema and type 2 diabetes	Low to medium: mainly focused animal models of colitis and in associative studies	Preclinical in mice and *in vitro*	18, 52, 53
Lactococcus lactis::elafin	GMO (host isolated from food)	Mainly inflammatory diseases such as IBD	Medium: good evidence from animal models of IBD	Preclinical in mice	20
Lactococcus lactis::trefoil factor 1 or IL-10	GMO (host isolated from food)	Allergen sensitivity and autoimmune diseases — type 1 diabetes	Medium: mainly animal-based efficacy	Human, phase 1 trial	23

O'Toole *et al.*, Nat Microbiol. 2017

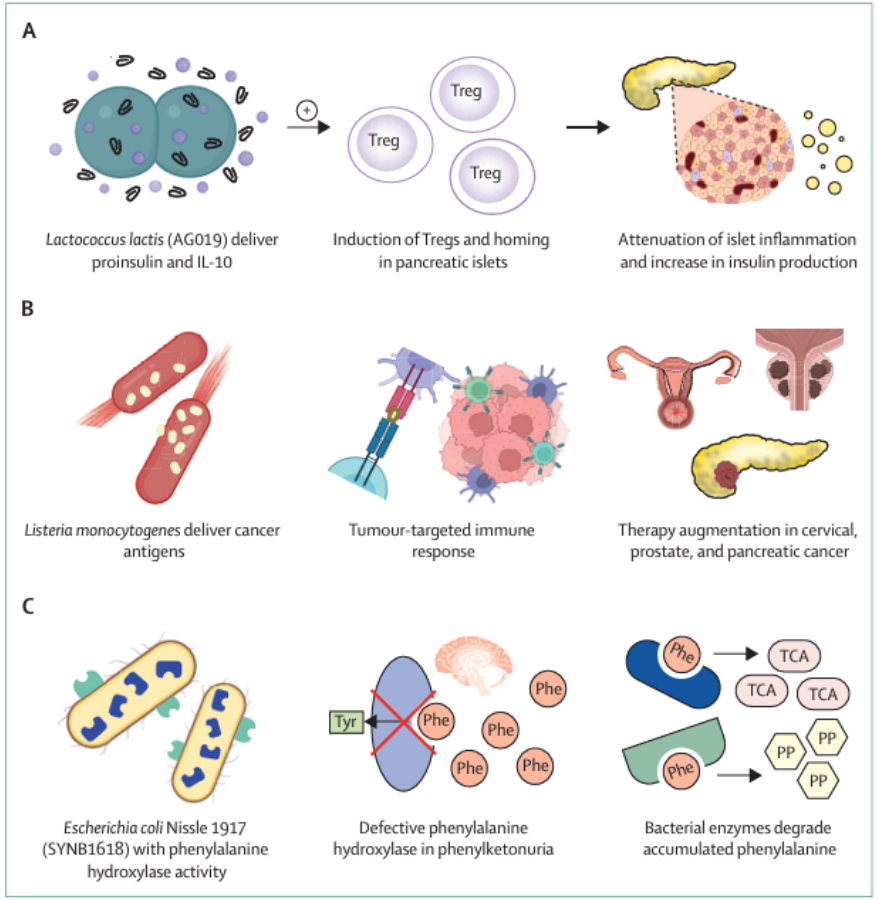
ENGINEERED COMMENSAL MICROBES FOR DIET-MEDIATED COLORECTAL-CANCER CHEMOPREVENTION – *E. coli* Nissle 1917

- *In vitro* models: >95% proliferation inhibition of murine, human and colorectal adenocarcinoma cell lines
- Murine models of colorectal carcinoma fed with engineered microbes and cruciferous vegetable diet: significant tumour regression and reduced tumour occurrence



Ho *et al.*, Nat Biomed Eng. 2018

ENGINEERED PROBIOTICS AS THERAPEUTIC CARRIERS



Porcari *et al.*, Lancet Gastroenterol Hepatol. 2024

- ✓ *Lactococcus lactis* delivering human proinsulin and IL-10, leading to the induction of Tregs and their homing to pancreatic islets (in early stages of T1D, this therapeutic alleviates autoinflammatory processes in pancreatic islets and improves insulin production)
- ✓ Attenuated live *Listeria monocytogenes* delivering tumour antigens for cancer vaccination (currently in clinical trials for adjuvant treatment of cervical, prostate, and pancreatic cancer)
- ✓ *Escherichia coli* Nissle 1917 expressing Phe-degrading enzymes, which can accumulate and cause severe neurological complications in people with phenylketonuria, due to defective conversion of Phe to Tyr by phenylalanine hydroxylase (the engineered probiotic carries enzymes capable of degrading Phe to the non-toxic metabolites phenylpyruvate and trans-cinnamate)

CONSENSUS STATEMENT ON THE DEFINITION AND SCOPE OF POSTBIOTICS

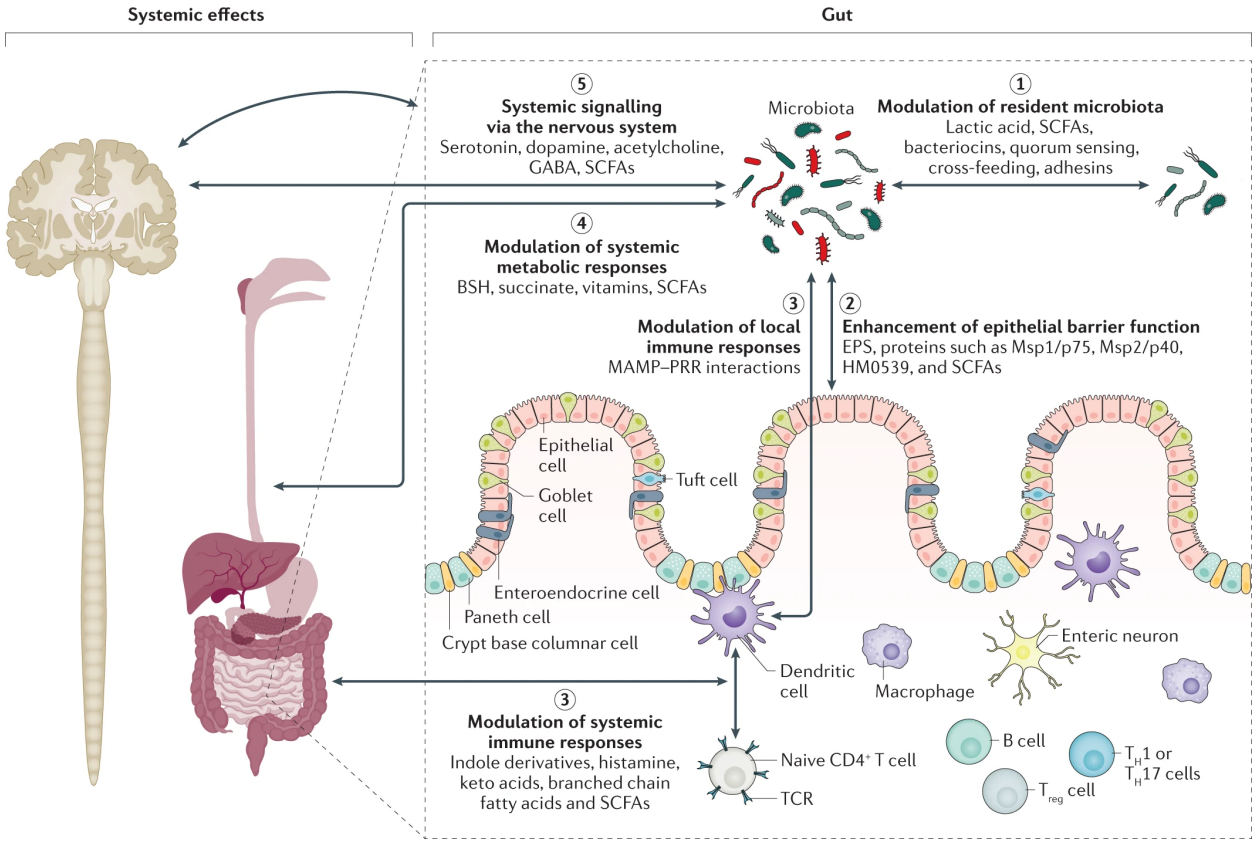
- ✓ Any factor resulting from the metabolic activity of a probiotic or any released molecule capable of conferring beneficial effects to the host in a direct or indirect way
- ✓ Soluble factors (products or metabolic byproducts), secreted by live bacteria, or released after bacterial lysis, such as enzymes, peptides, teichoic acids, peptidoglycan-derived muropeptides, polysaccharides, cell surface proteins and organic acids
- ✓ Compounds produced by microorganisms, released from food components or microbial constituents, including non-viable cells that, when administered in adequate amounts, promote health and well-being
- ✓ Non-viable metabolites produced by probiotics that exert biological effects on the hosts
- ✓ Non-viable bacterial products or metabolic byproducts from probiotic microorganisms that have positive effects on the host or microbiota
- ✓ Functional bioactive compounds, generated in a matrix during fermentation, which may be used to promote health

"Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" (Salminen *et al.*, Nat Rev Gastroenterol Hepatol. 2021)

CONSENSUS STATEMENT ON THE DEFINITION AND SCOPE OF POSTBIOTICS

Criteria for a preparation to qualify as a postbiotic

- Molecular characterization of the progenitor microorganisms (e.g., fully annotated genome sequence) to enable accurate identification and screen for potential genes of safety concern
- Detailed description of the inactivation procedure and the matrix
- Confirmation that inactivation has occurred
- Evidence of a health benefit in the host from a controlled, high-quality trial
- Detailed description of the composition of the postbiotic preparation
- Assessment of safety of the postbiotic preparation in the target host for the intended use

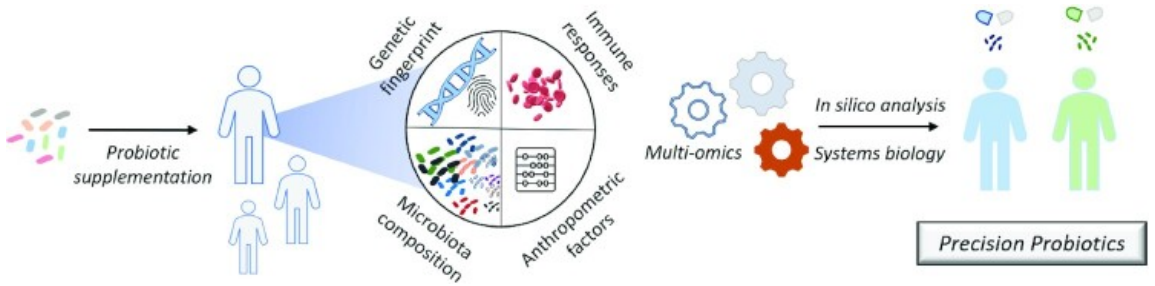


Salminen *et al.*, Nat Rev Gastroenterol Hepatol. 2021

… TOWARDS PERSONALIZED PRECISION PROBIOTIC THERAPY

Critical issues & open questions:

- **Host characteristics** (genetics, clinical parameters, diet, lifestyle, etc.)
- **Microbiome characteristics** (compositional and functional profile, including networks)
- **Dosage** (ranging from 1×10^{8} to 1.8×10^{12} CFU twice daily depending on strain and disease, based on at least 1 well-designed clinical trial showing a beneficial effect for a health-promoting or therapeutic outcome - International Scientific Association for Probiotics and Prebiotics)
- **Duration** (1 month?)
- **Time of administration** (before, during or after meals?)
- **Matrix**
- **Single vs. multi-strain formulations**
- **Mechanisms of action** (postbiotics?)



Kiousi *et al.*, Adv Nutr. 2021