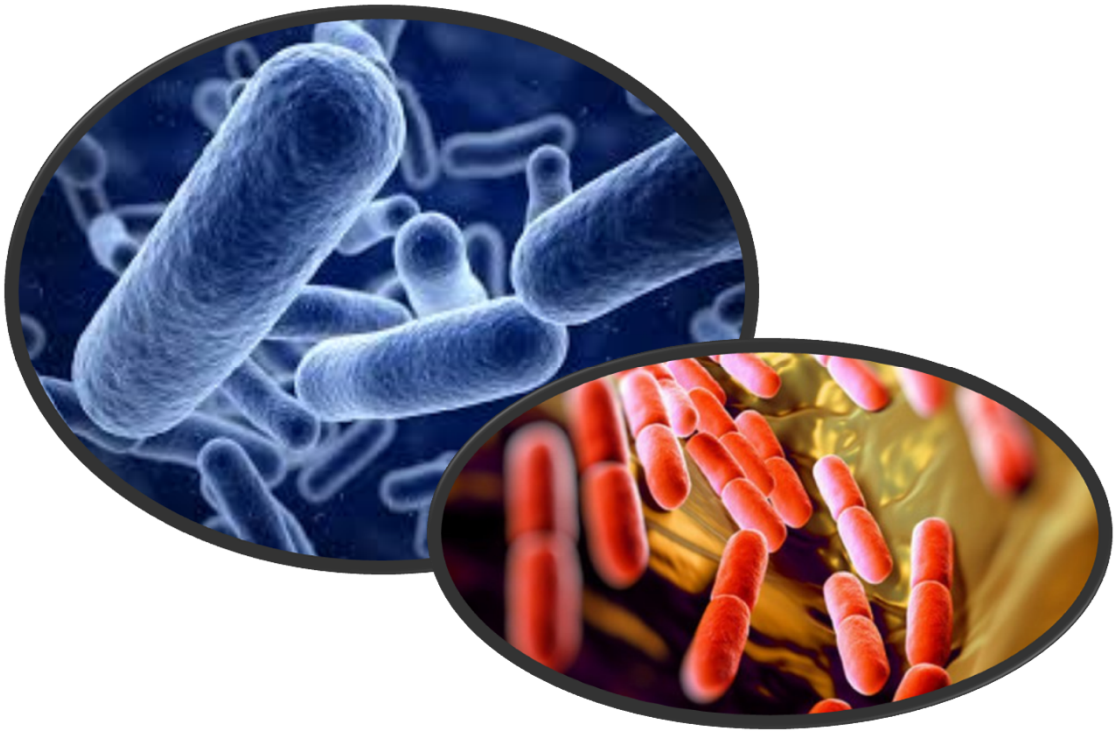
Designing Next-Generation Probiotics and Prebiotics for Improved Human Health



Rina Agustina

Department of Nutrition

Faculty of Medicine, Universitas Indonesia; Dr. Cipto Mangunkusumo Hospital

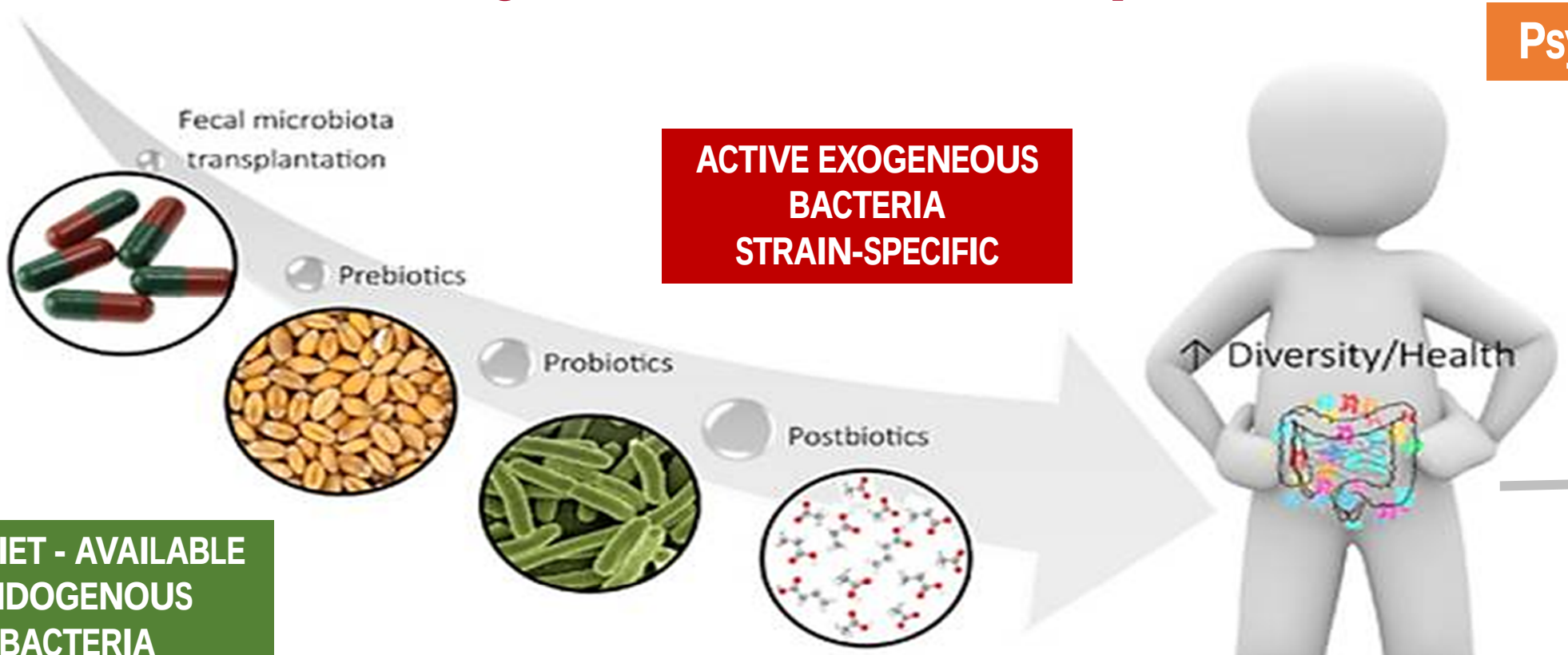
Human Nutrition Research Center, IMERI Jakarta, Indonesia

ILSI INDIA CONFERENCE DECEMBER 6, 2018

OUTLINE

- Potential strategies on modulating gut microbiota composition
- ‘Current Generation’ of Probiotics
- Advances Metagenomics
- Next Generation of Probiotics versus Life Bacterio-therapeutic product
- Novel Prebiotics
- Technical, Research and Regulatory challenge
- Conclusion

Potential strategies aimed at modulation of the gut microbiota composition



ACTIVE EXOGENEOUS BACTERIA STRAIN-SPECIFIC

IA DIET - AVAILABLE ENDOGENOUS BACTERIA

Metabolites

Vieira, et al 2016

Psychobio

Nutribiotic

Pharmabi

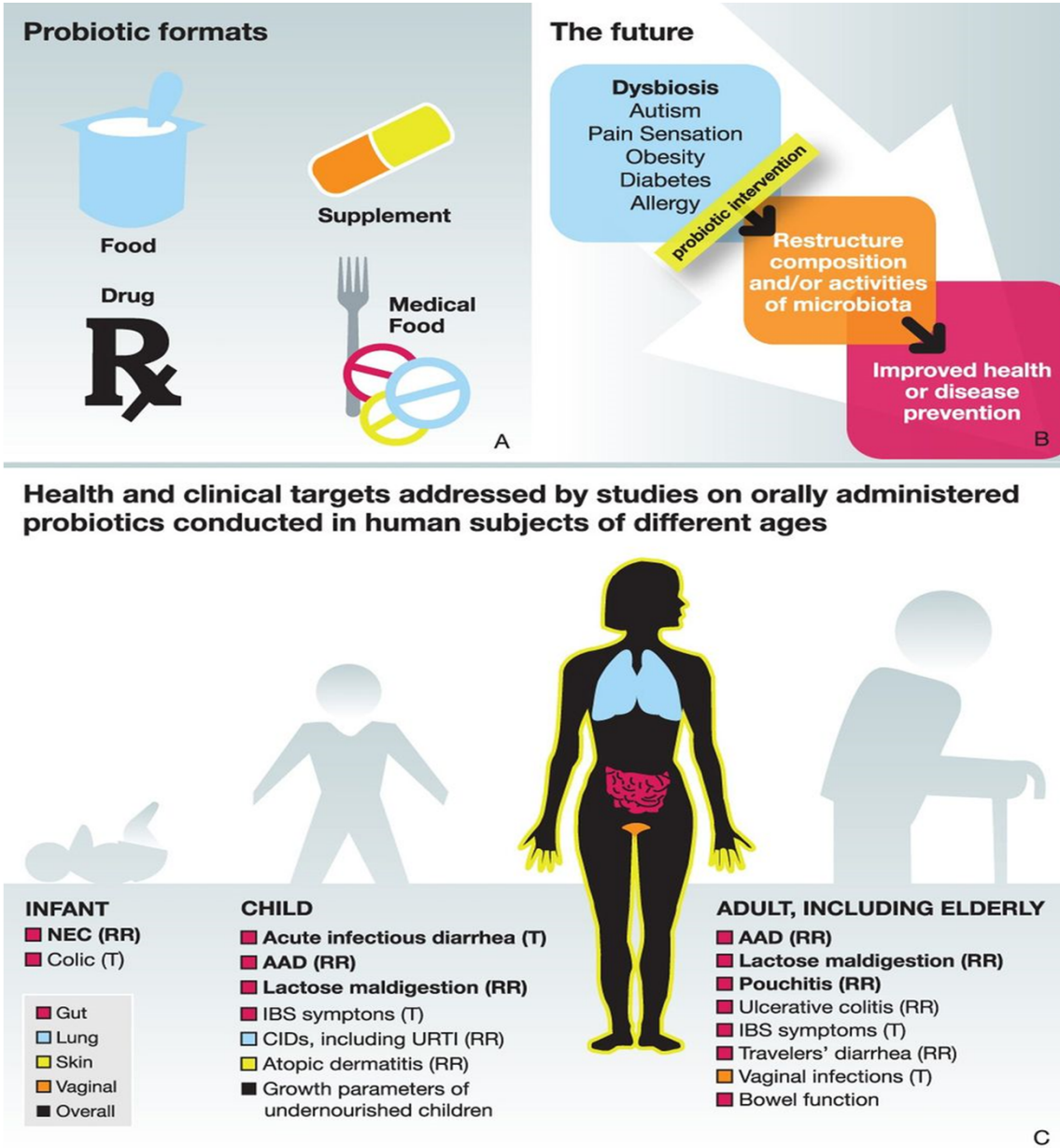
CURRENT PROBIOTIC EFFICACY

ome probiotics and prebiotics are suggested to lay roles in alleviating disease symptoms, rotection against infections, inflammatory bowel iseases, and atopic diseases, modulating the mmune system, obesity and neurological roblem by improving the beneficial gut icrobiota colonization.

he best documented clinical areas for probiotics:

- Treatment of acute gastroenteritis in children
- Prevention of antibiotic-associated diarrhea both in children and in adults

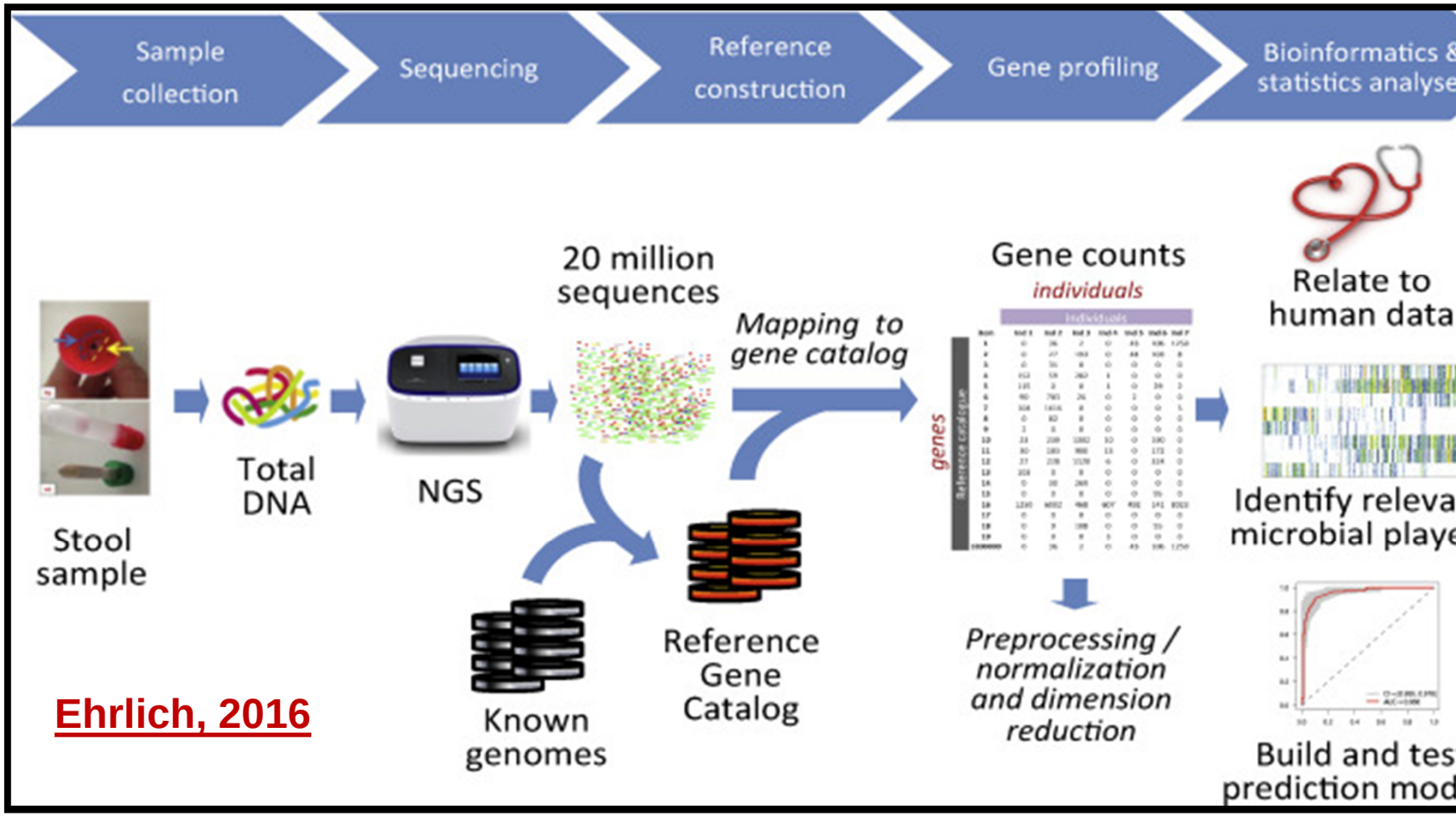
owever, the main existing probiotics to onsumers are generally produced from a limited ange of organisms (genera Lactobacillus – ifidobacterium).



Sanders, BMJ, 2012

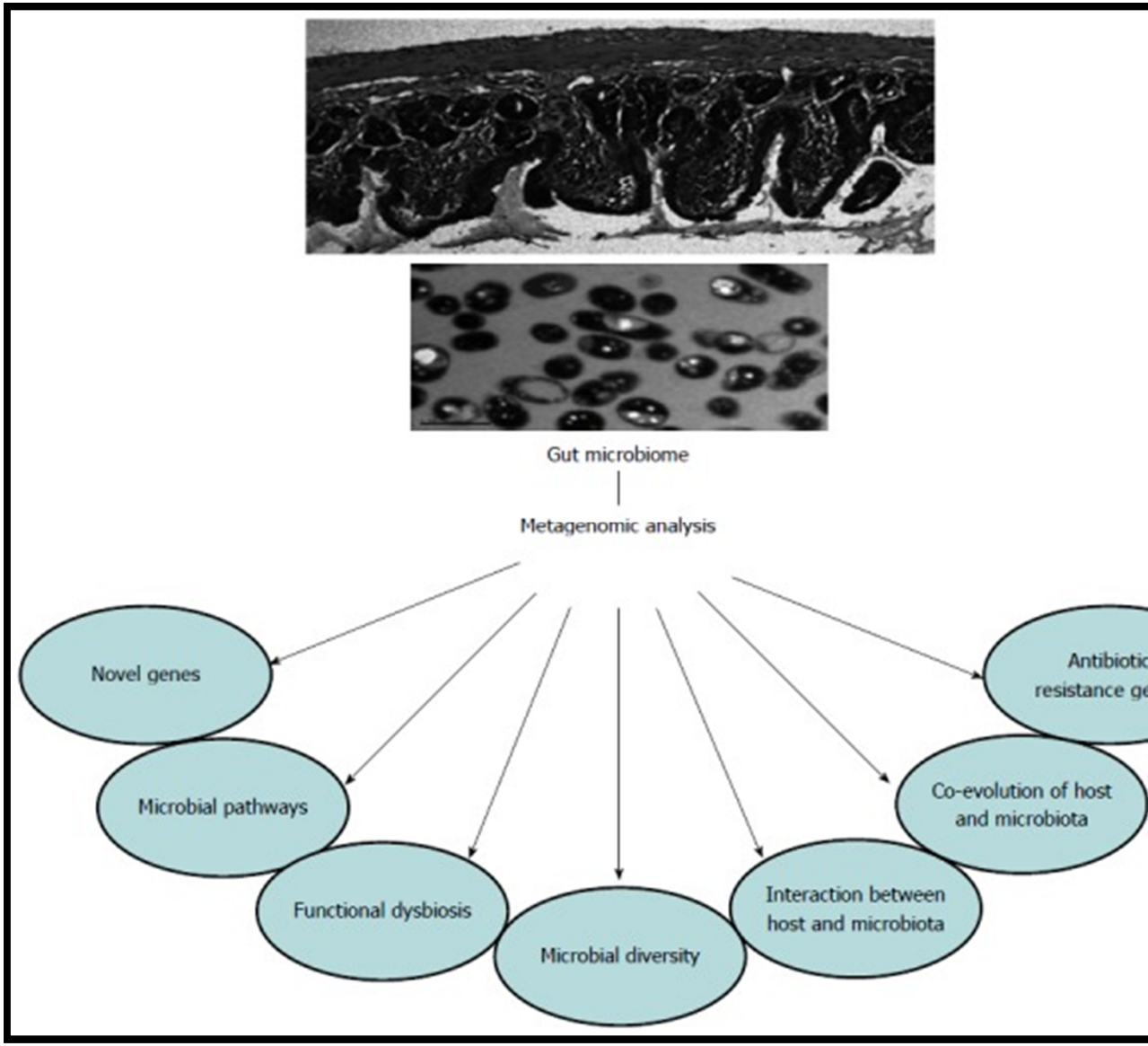
Metagenomics approach and bio-informatics analyses

- have provided the opportunity to study complex microbial communities and
- help ascertain general patterns that regulate human microbial ecosystems.



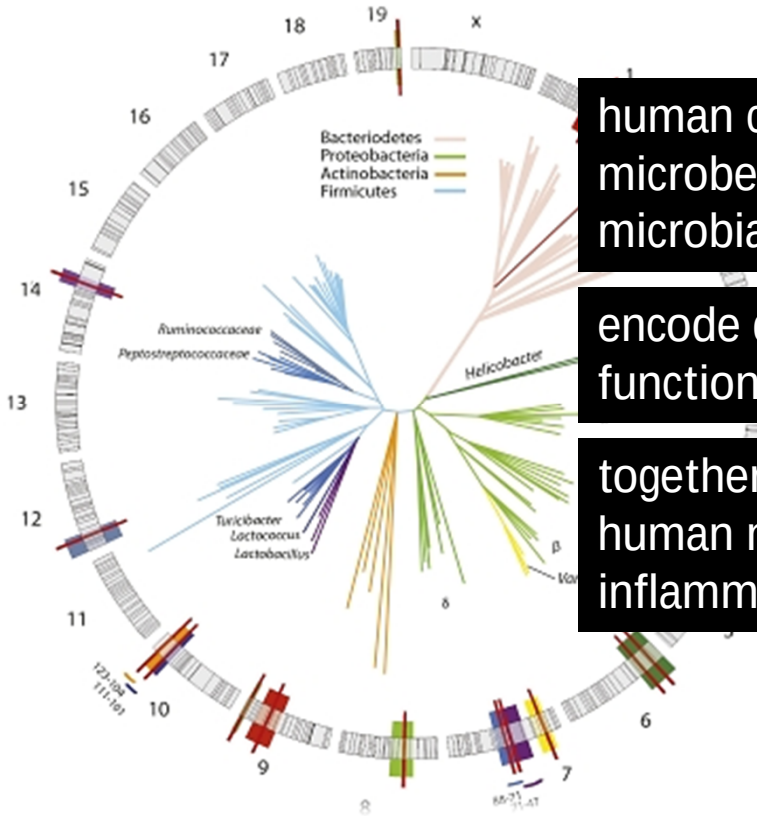
Metagenomics approach and bio-informatics analyses

With this advancement, the discovery of new-generation probiotics and prebiotics will continue to evolve in modulating the intestinal and extra-intestinal health.



GUT MICROBIOTA "A COMPLEX COMMUNITY"

high complexity and (interpersonal) diversity



human cells and microbes, human a microbial genome

encode diverse functions and pathv

together affecting human metabolic a inflammatory featu

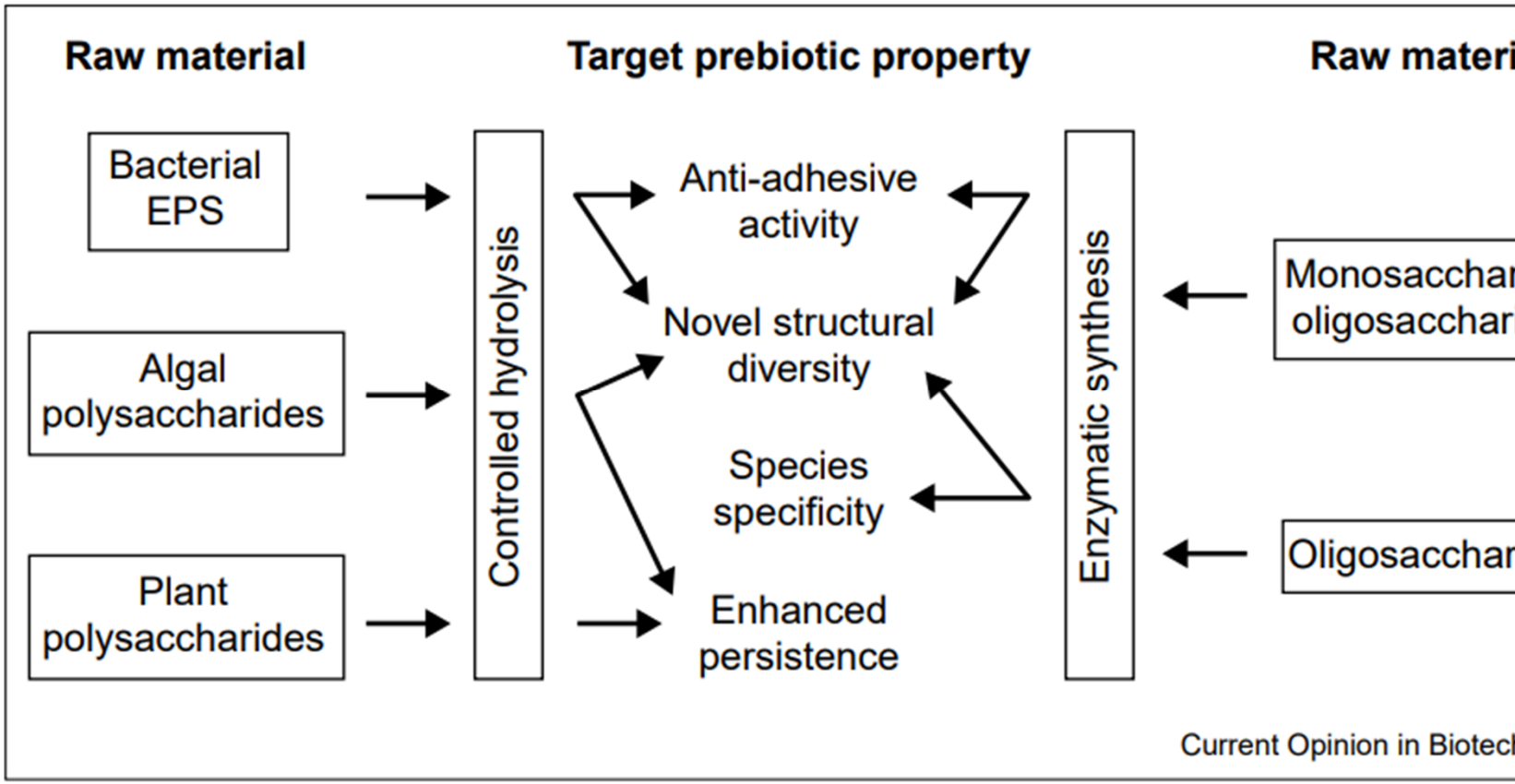
In the population
•1100 species
•10 mil genes

▪In each individual
•At least 160 species
•Approx 540k genes – we share almost 40% of these genes with any other person

Benson, 2010; Buccigrossi
Sommer & Bäckhed, 2

Biotechnological manufacture of enhanced prebiotics

- At the same time, new manufacturing biotechnologies for prebiotics, and better understanding on the oligosaccharides metabolism by probiotics
- are facilitating the development of prebiotics and synbiotics for specific functional properties and health outcomes.



Rastall and Maitin

Why Next-generation Probiotic and Prebiotic?

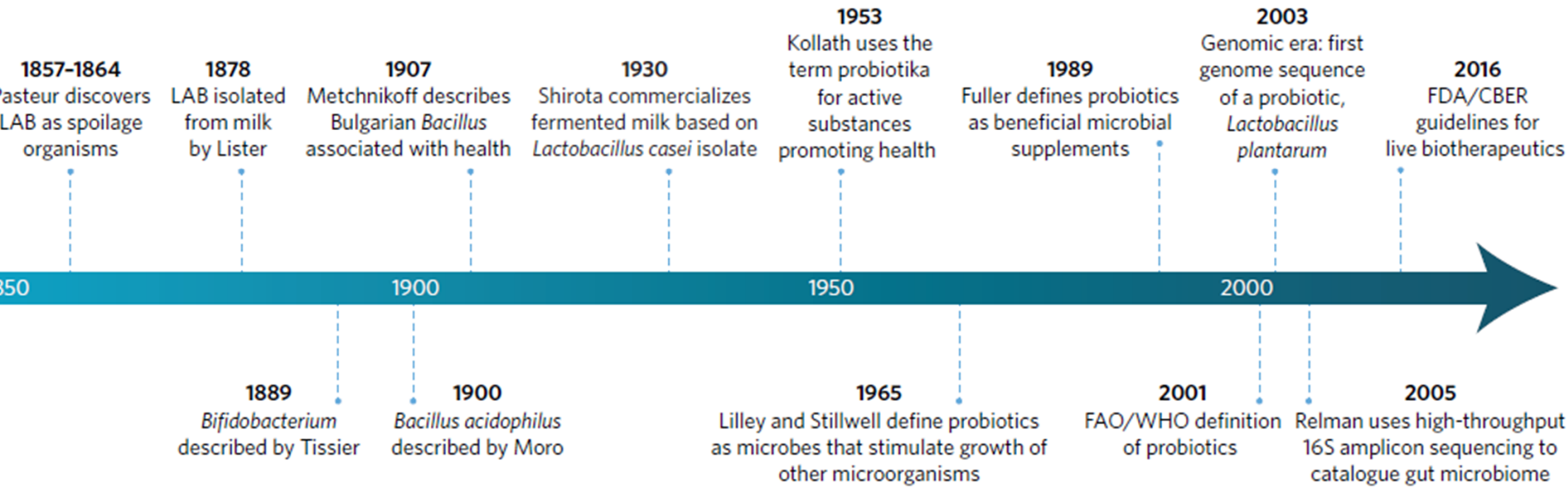
Probiotics

- Limited list of genera, which mainly include Lactobacillus spp. and Bifidobacterium spp.
- The main existing probiotics to consumers are generally produced from a limited range of organisms, mainly sourced from the gut or from traditional fermented foods, such as pickles, yoghurts and kefir grains.

Prebiotics

- Several commercial prebiotics (or as synbiotics) products worldwide such as soybean oligosaccharides raffinose and stachyose, glycanases, Arabinogalacto-oligosaccharides, Pectic oligosaccharides Gentio-oligosaccharides or other, need to be thoroughly tested, that can be potential for development of novel prebiotics

Timeline of selected milestones in the history of probiotics and next-generation probiotics



ure 1 | Timeline of selected milestones in the history of probiotics and next-generation probiotics. LAB, lactic acid bacteria.

O'Toole, P., Marchesi, J., & Hill, C. (2017). Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature Microbiology*, *2*(5). doi: 10.1038/nmicrobiol.2017.57

Probiotics

FAO and WHO definition **In 2001**

Probiotics: ***"Live microorganisms which when administered in adequate amounts, confer a health benefit on the host"***

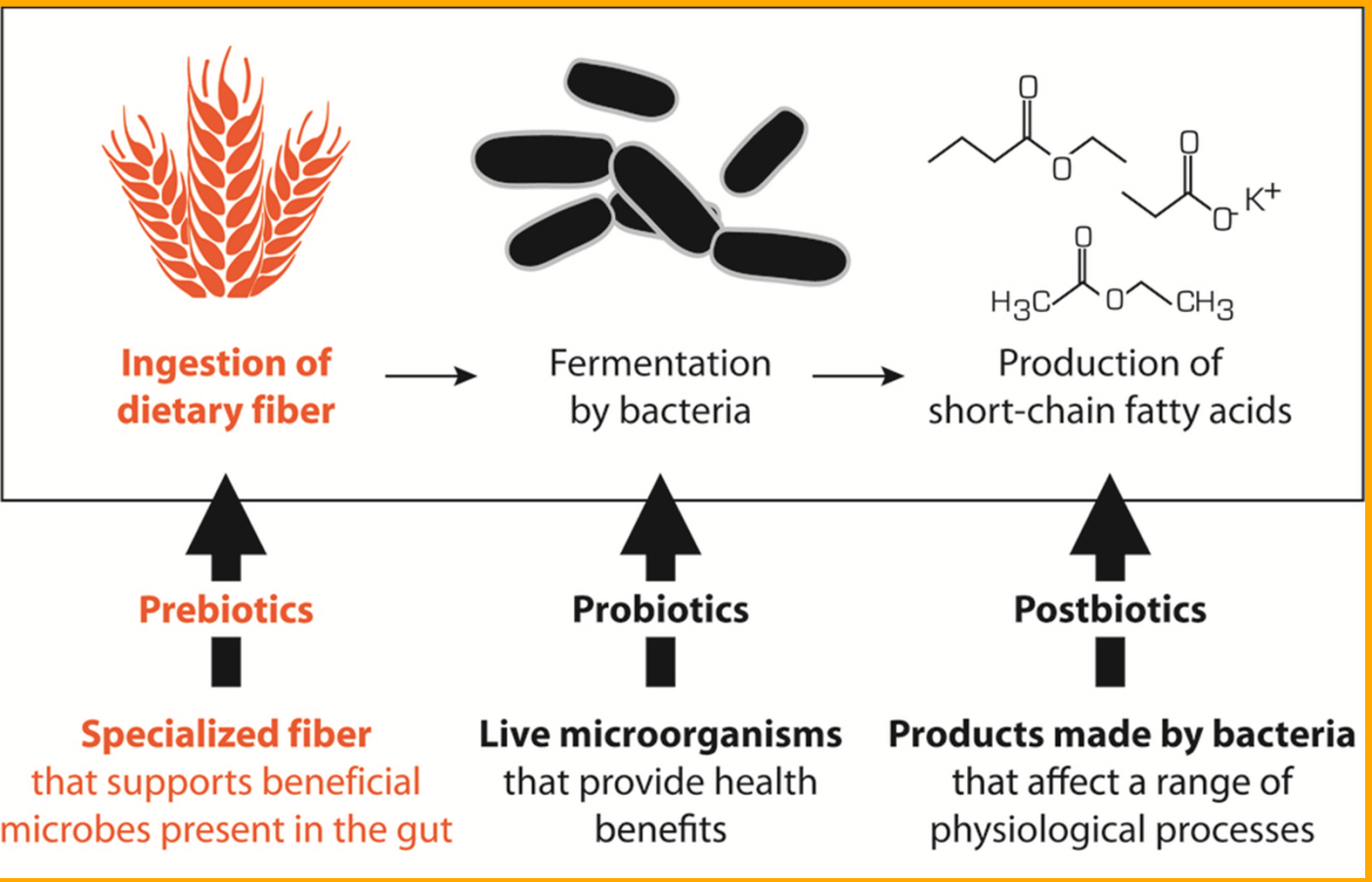
Essential: Microbial, Viable, Beneficial to health and **Strain-specific**

The active bacteria are mostly *Lactobacillus* and *Bifidobacteria*

The global probiotics market is projected to reach a turnover value of US$46.55 billion by 2020

(http://www.marketsandmarkets.com/PressReleases/probiotics.asp)

Prebiotics



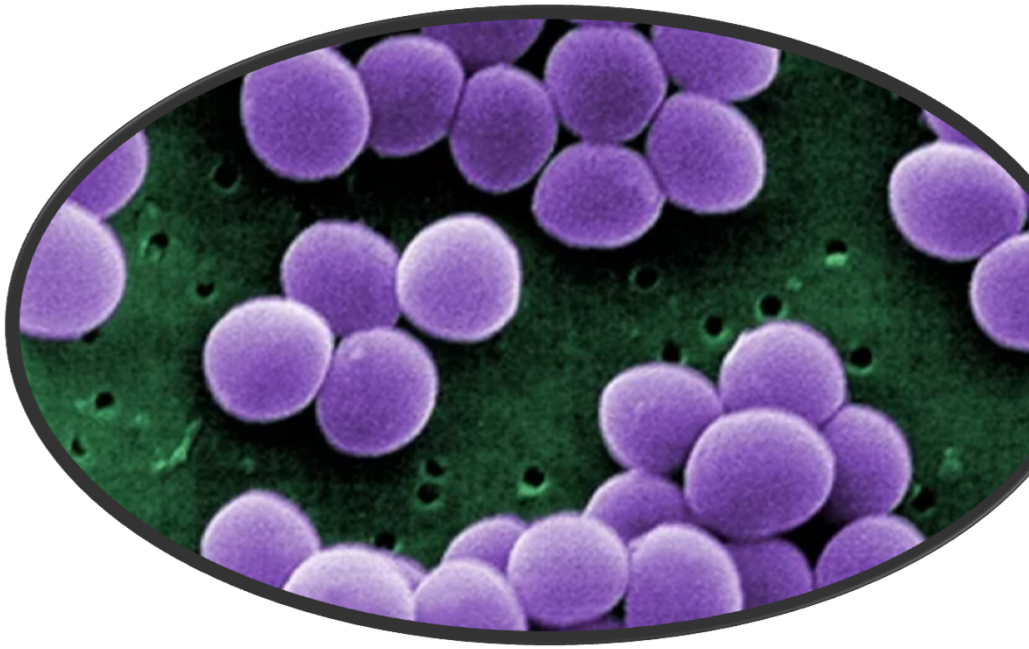
"nondigestible oligosaccharides, such as fructooligosaccharides, galactooligosaccharides, lactulose, and inulin, which have the potential to stimulate growth of selective and beneficial gut bacteria, particularly lactobacilli and bifidobacteria"

Gibson and Roberfroid, 1995

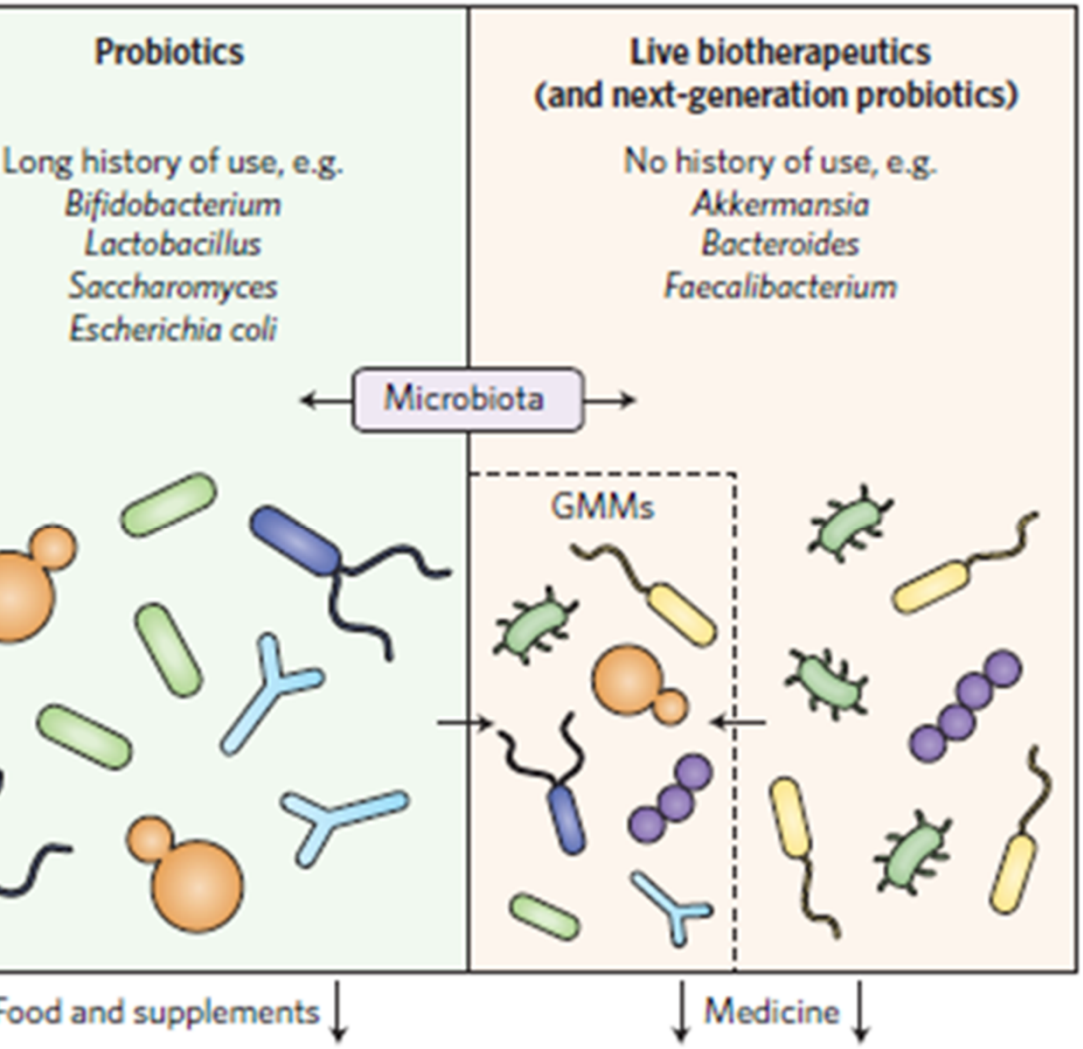
fructooligosaccharides, galactooligosaccharides, lactulose, and inulin

Next-generation Probiotics (NPGs)

Defined as is with the WHO-FAO definition of a probiotic, with an extension of understanding to primarily referring to those microorganisms that have not currently been used as agents to promote health especially in a pharmaceutical product, than a food delivery route
Non-traditional microorganisms



Next - Generaton Probiotics (NGPs) versus Live Biotherapeutic Products (LBPs)



The US Food and Drug Administration (FDA) definition of a LBP: "a biological product that:

1. Contains live organisms, such as bacteria
2. is applicable to the prevention, treatment, cure of a disease or condition of human beings
3. is not a vaccine".

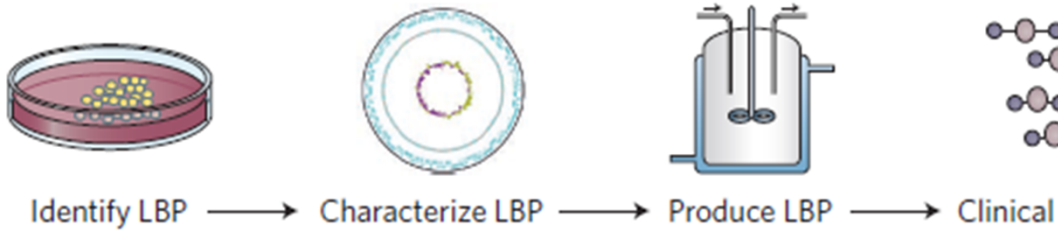


Figure 3 | Graphical summary of the pathway to regulatory approval live biotherapeutic products.

Selected examples of next-generation probiotics

Organism	Type	Disease target	Level of evidence	Study type
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
Bacteroides ovatus D-6	Natural (human)	Cancer	Low to medium: increases levels of murine TFα-specific IgM and IgG	Preclinical in mice
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
Bacteroides ovatus V975	GMO expressing TGF-β1	Intestinal inflammation	Medium: shows abrogation of symptoms of DSS induced in murine colitis model	Preclinical in mice
Bacteroides dorei D8	Natural (human)	Heart disease	Low: depletion of cholesterol *in vitro*	Preclinical *in vitro*

(O'Toole, P., Marchesi, J., & Hill, C., 2017).

Selected examples of next-generation probiotics

Organism	Type	Disease target	Level of evidence	Study type
Bacteroides fragilis ZY-312	Natural (human)	Clearance of infectious agents	Low: data only *in vitro*	Preclinical *in vitro*
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
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
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*
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
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

(O'Toole, P., Marchesi, J., & Hill, C., 2017).

ext Generation obiotic: *Akkermansia uciniphila* as a utative candidate?

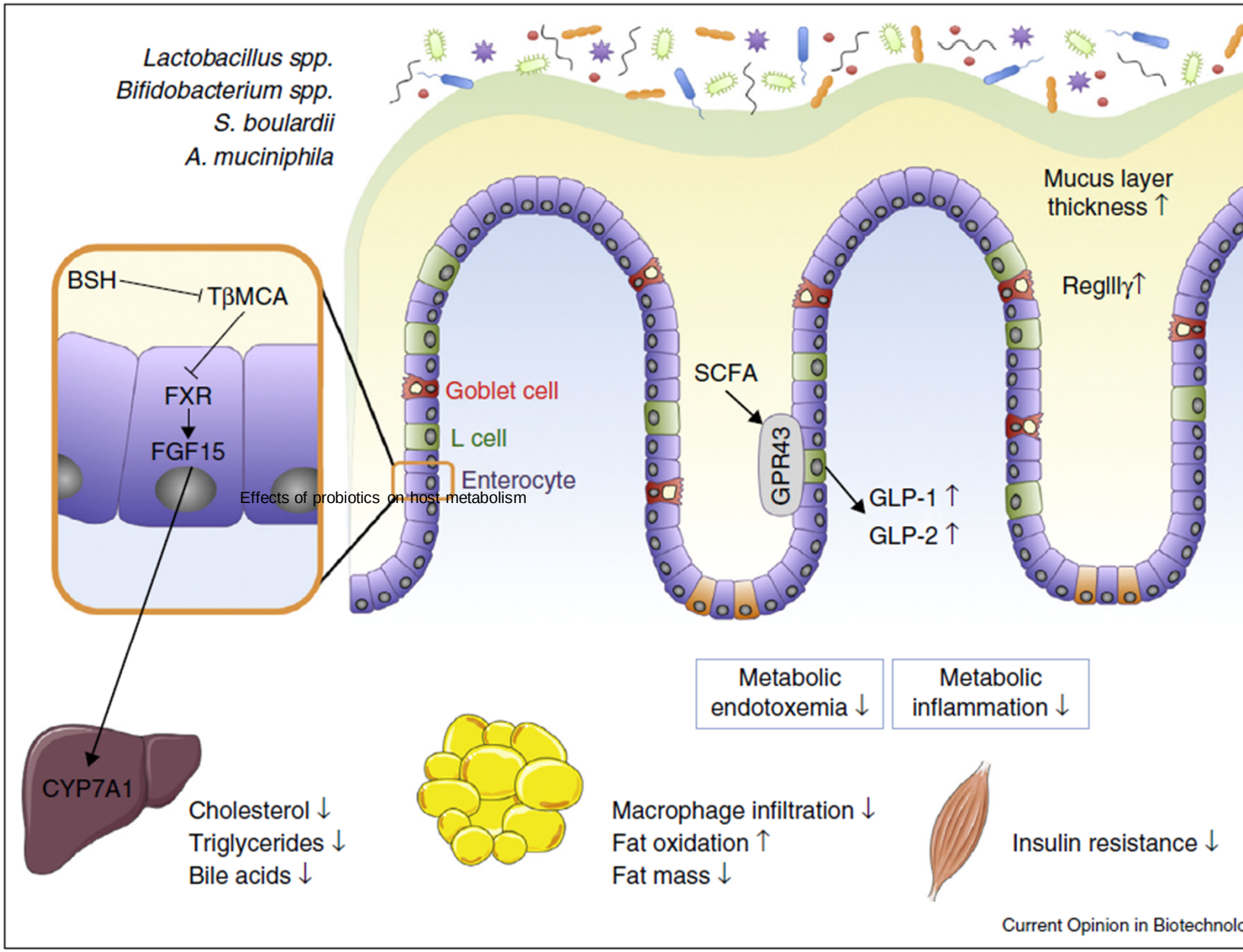
ani, P., & Van Hul, M. 018).

ovel opportunities for ext-generation obiotics targeting etabolic syndrome.

- Fei and Zhao reported than the *Enterobacter cloacae* B29 strain isolated from the gut microbiota of an obese human induced obesity in germ-free mice
- Conversely, *A. muciniphila*, a mucin-degrading bacterium that resides in the mucus layer, is present at lower levels in obese and type 2 diabetic subjects
- Prebiotic treatment (i.e., oligofructose) increased the abundance of A. muciniphila in obese mice → resulted in an improved metabolic profile.
- *A. muciniphila* treatment decreased metabolic endotoxemia and adipose tissue inflammation by improving intestinal mucosal barrier function at different levels.

Effects of probiotics on host metabolism

Cani, P., & Van Hul, M. (2018).



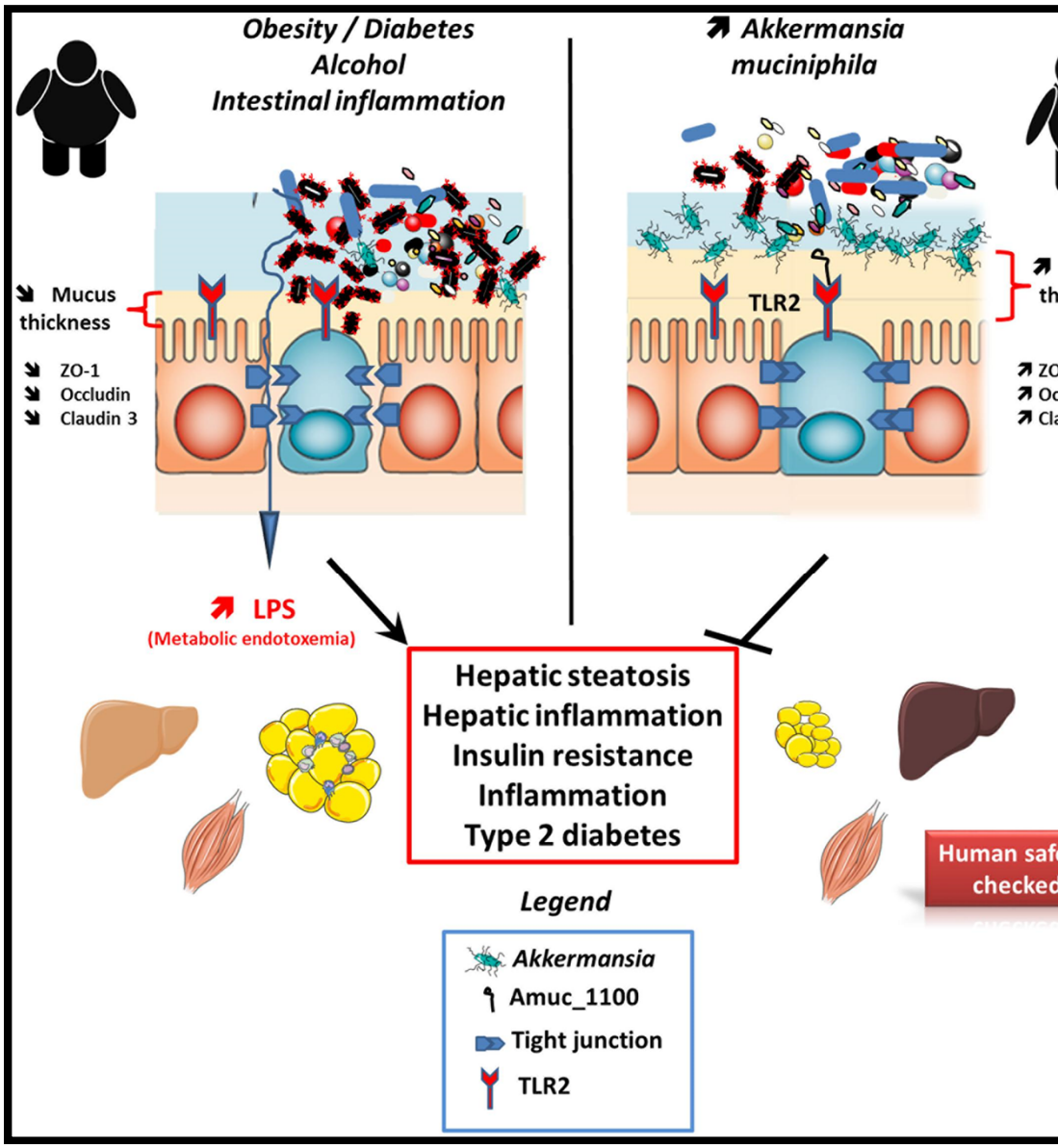
Effects of probiotics on host metabolism

lext-Generation Beneficial Microbes:

kkermansia muciniphila
n host metabolism

trice D. Cani[1*] and Willem M. de Vos

A. muciniphila has been found to be lower in several conditions such as during obesity, diabetes, intestinal inflammation, liver diseases, or chronic alcohol consumption.
This is associated with an altered gut barrier function leading to an increased plasma LPS levels and eventually triggering low grade inflammation and metabolic disorders.
A. muciniphila alive or pasteurized as well as Amuc_1100 has been shown to restore gut barrier function likely by acting on TLR2 and restoring appropriate tight junction expression.
All these results are associated with an increased mucus later thickness and an improvement of metabolic disorders. It is worth noting that an exploratory human investigation has shown that *A. muciniphila* is apparently safe.



Next Generation Probiotic: Novel Strain?

Faecalibacterium prausnitzii

- *F. prausnitzii* may possess in vivo and in vitro anti-inflammatory effects.
- Able to reduce Th1 and Th17 proinflammatory cytokine in Mesenteric Lymphatic Node (MLN) and colon tissues both DNBS and DSS colitis model.
- Can induce the Clostridium-specific IL-10-secreting regulatory T cell subset, present in several human colon cells.
- Its capacity for lowering IL-12 and IFNγ production indicates that the interaction between *F. prausnitzii* and the host shape and maintain the gut barrier immune function (Quévrain et al., 2016).

Breyner et al. (2017). Microbial anti-inflammatory molecule (MAM) from Faecalibacterium prausnitzii shows a protective effect on DNBS and DSSinduced colitis model in mice through inhibition of NF-κB pathway.

ext Generation obiotic: Novel train?

cteroides fragilis and *cteroides uniformis*

- B. fragilis produces polysaccharide A (PSA), an immunomodulatory molecule that activates the T-cell dependent immune responses (Troy and Kasper, 2010). It involved in the development and homeostasis of the host immune system *(Troy and Kasper, 2010).*
- Oral administration of Bacteroides uniformis (B. uniformis) CECT 7771 in high fat diet-fed mice improved lipid profile, reduced glucose insulin and leptin levels, increased TNF-α production by dendritic cells (DCs) in response to LPS stimulation, and increased phagocytosis *(Gauffin Cano et al., 2012)*
- It can ameliorate metabolic disorder and immunological dysfunction related to intestinal dysbiosis in obese mice *(Gauffin Cano et al., 2012; Yang et al., 2016).*

robiotics on central nervous system (CNS) disorders:
ildren with autism, anxiety and depression

n association between **hifts in the gut microbiota omposition in children with neurodevelopmental isorders** (e.g. autism pectrum disorder, anxiety nd depressive behaviors)

emporal profile of eurodevelopmental equences in relation to the ge of onset of mental isorders and degree of icrobiota tability/diversity roughout life

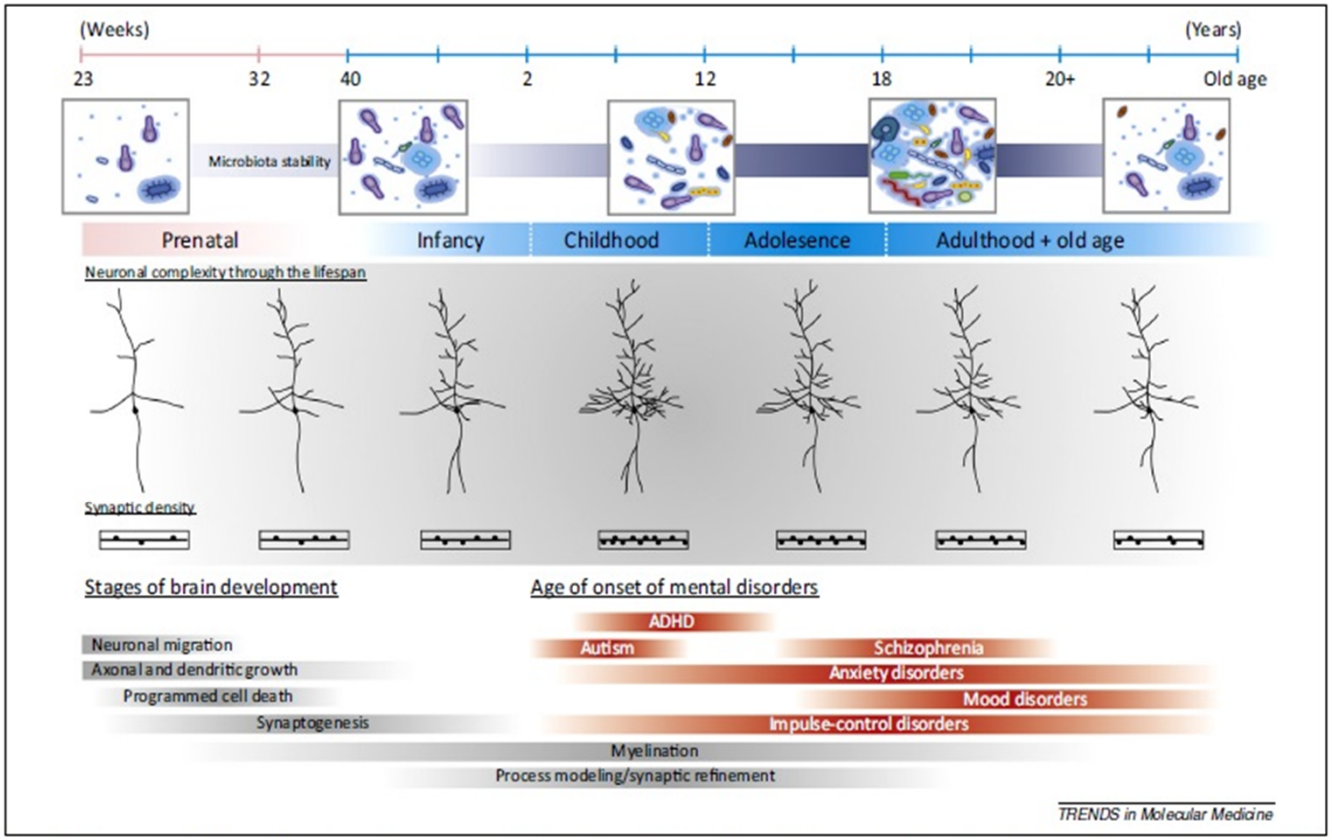


Figure 2. Temporal profile of neurodevelopmental sequences in relation to the age of onset of mental disorders and degree of microbiota stability/diversity throughout life. Gut microbiota is essential to human health and is a key player in the bidirectional communication between the gastrointestinal tract and the central nervous system. The microbiota dynamically changes throughout lifespan, establishing its symbiotic rapport with the host with critical windows during infancy, adolescence, and aging. During these windows, the organism is vulnerable to external stressors, which may result in mental disorders. Early life perturbations of the developing gut microbiota can impact neurodevelopment and potentially lead to adverse mental health outcomes later in life. The concept of parallel and interacting microbial–neural critical windows opens new avenues for developing novel microbiota-modulating based therapeutic interventions in early life to combat neurodevelopmental deficits and brain disorders. Abbreviation: ADHD, attention deficit hyperactivity disorder.

e Lancet 2018: sequencing
nes – 18 genes candidates

Borre *et al.*, 2014

Technical Challenges

- Stability of the probiotic during the probiotic production → The product manufacturing and storage processes may impact the viability of the bacterial strains, influencing probiotic stability and properties.
- Viability of the probiotics after consumption → Bacterial strains should remain viable at sufficient numbers through the gastrointestinal tract (GIT) passage → the selection of optimal culture medium and cell protectants is crucial

Research challenges and opportunities

- Strategy of how scientists may meet this challenge in designing the NPGs with new strainsfrom the lessons learned working with traditional probiotics, and prebiotics.

Importantly, series of clinical trials (Phase 1 for safety and dosage ranges; Phase 2 will for the primary endpoint in a small groups; preferably the Phase 3 for evaluating the efficacy, side effects and relative benefits in a larger group) need to be determined in general use or for vulnerable populations.

Regulatory Challenges

The definition and classification of probiotics by regulatory agents throughout the world is different → the status of probiotic products is still uncertain. How the regulation of new product of NGPs will align with LBPs

Thus, reservations about probiotic products claims may arise among regulatory bodies, producers, and consumers.


THANK YOU