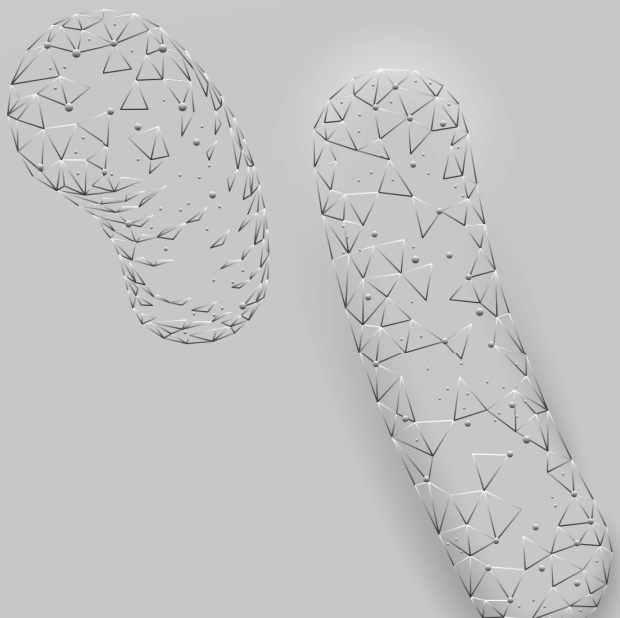
CHAPTER 1.

Introduction



1.1 THE HUMAN MICROBIOTA

1.1.1 Colonization by microbes

Bacteria, archaea, protozoa, fungi and viruses colonize the entire human body. It is estimated that these microorganisms outnumber the human cells 3:1, and that the combined microbiota may weigh up to five pounds (Sender et al., 2016). Microbes can be found in our oral cavities, skin, urethra, bladder, placenta, lungs and biliary tracts, but the vast majority (70%) colonize the human gastrointestinal tract (GIT). Particularly the small intestine and colon, as these have a relatively large surface area (>200M^2) and provide an abundance of nutrients for microbial growth (Ley et al., 2006b). It is projected that the GIT harbours over 2000 different bacterial species and over 10,000 bacterial strains in the combined human population (Almeida et al., 2019). While some of these bacteria can be pathogenic (either inherently or through their metabolites), most are commensal or have a mutualistic relationship with their host and therefore live in peaceful coexistence (Quigley et al 2013).

1.1.2 Gut microbiota composition

The collection of all colonizing microbes is referred to as the human microbiota, which can be classified per phylum, class, order, family, genus, and species. The microbial phyla Firmicutes and Bacteroidetes represent 90% of the gut microbiota. Within the Firmicutes phylum, *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminicoccus Clostridium* are the predominant genera. Bacteroidetes consist predominantly of the genera *Bacteroides* and *Prevotella* (Rinninella et al 2019).

1.1.3 Personal microbiota

While it was initially thought humans are born sterile (Gareau et al 2010), recent studies indicate that the foetus is exposed to some commensal bacteria in utero from the maternal gut which cross the placenta and infiltrate the amniotic fluid (Isolauri et al., 2017). This exposure to colonizing bacteria continues upon birth and throughout the first year of life and has a profound influence on lifelong health. Delivery through the vaginal tract and skin-to-skin contact are vital exposures of an infant to complex microbial communities which form an integral part of the infant's microbiota later in life (Ley, Peterson & Gordon 2006a). This is underlined by the fact that infants delivered through caesarean section have contrasting microbiota compositions compared to those delivered through the vaginal tract (Ravel et

al., 2011). Additionally, monozygotic twins have a similar microbiota composition compared to that of their other siblings, suggesting that colonization by maternal microbial communities is more influential in the microbiota formation than host genotypes (Turnbaugh et al., 2009; Turnbaugh et al 2010). After the initial formation of the microbiota and during the first life year, the composition has low diversity but varies widely between individuals and with time (Rodríguez et al 2015). After the first year, the microbiota becomes more stable and shows relatively little temporal variability into adulthood. Around the elderly age, the microbiota decreases in diversity again and becomes more susceptible to change (Rodríguez et al 2015). Throughout life, host and environmental factors, such as diet, medication usage (in particular antibiotics), physical activity, smoking habits and disease, have a strong impact on the composition and diversity of the microbiota (Wen & Duffy, 2017). Figure 1.1 shows an overview of the endogenous microbiota throughout life and the impact of external factors on its composition.

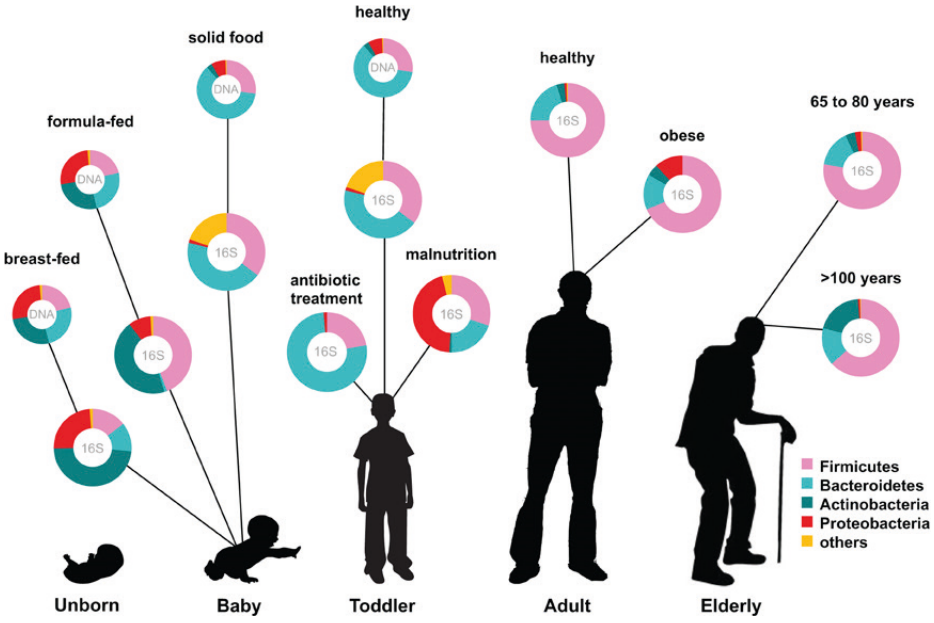


Figure 1.1 Microbiota composition & diversity throughout life.

This Figure illustrates how the gut microbiota composition changes with age and is affected through external host and environmental factors (e.g. antibiotic treatment). By Ottman et al (2012).

1.1.4 Mutualistic relationships

The gut microbiota is vital to human health and wellbeing as the mutualistic microorganisms within it perform functions that are known to be beneficial. While much remains to be determined about its exact role and mechanism of action, it is frequently reported that the endogenous microbiota may support the host by:

- Protecting the host against pathogens by: (1) competing for binding sites and nutrients, (2) stimulating the host's antimicrobial compound production, (3) and producing bacteriocins that inhibit the growth of similar or closely related bacterial strains (Kamada et al., 2013).
- Stimulating metabolism and nutritional intake by fermenting dietary carbohydrates into short chain fatty acids (SCFA), such as butyrate, propionate and acetate. These are rich sources of energy that regulate the production of lipids and vitamins, increase colonic pH levels, improve gut integrity, alter cell proliferation, and increase anti-inflammatory, antitumorigenic, and antimicrobial functioning (Macfarlane & Macfarlane (2003); Sartor 2008; Byrne et al 2015; Tan et al 2014).
- Maintaining structural integrity and functioning of the GIT through the: (1) expression of small proline-rich protein 2A (sprr2A) which is required to maintain desmosomes at the epithelial villus (Lutgendorff et al 2008), (2) stimulation of TLR2 mediated signalling which maintain the tight junction functioning (Cario et al., 2007), (3) production of soluble proteins (p40 and p75) that can prevent cytokine induced apoptosis of intestinal epithelial cells (Yan et al., 2011), (4) stimulation of endocannabinoids that control gut barrier functions by decreasing metabolic endotoxemia (Cani et al., 2009), and (5) induction of the transcription factor angiogenin-3 which benefits the development of intestinal microvasculature (Stappenbeck et al., 2002).
- Altering immunomodulatory functioning in tandem with innate and adaptive immune systems. Gut associated lymphoid tissues (GALT), IgA producing B cells, innate lymphoid cells, effector and regulatory T cells, and resident macrophages and dendritic cells are regulated by the gut microbiota in various manners, of which the exact mechanism often remains to be determined (Jandhyala et al 2015).
- Modulating brain chemistry and neuro-endocrine systems through the Gut-Brain-Axis (GBA), the bi-directional biochemical signalling that takes place between the GIT and the central nervous system. Gut microbes can communicate with the GBA through the production of neuroactive and neuroendocrine

molecules such as epinephrine, norepinephrine, serotonin and histamine, thereby regulating anxiety, stress response and memory functioning (Carabotti et al., 2015; Forsythe et al., 2010; Bienenstock et al., 2010).

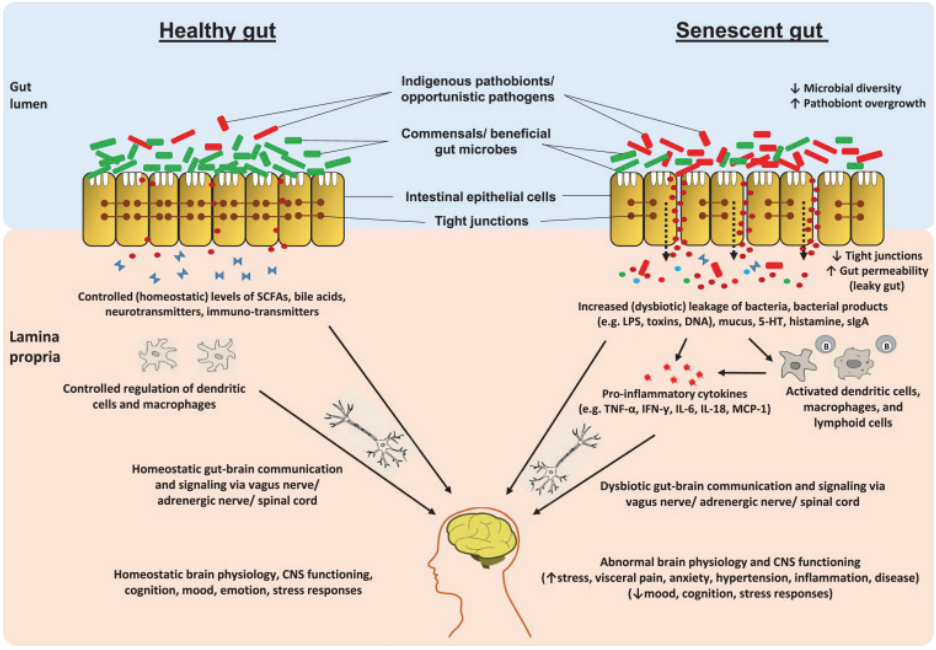


Figure 1.2 A senescent gut is a risk factor for disease: an example mediated by the gut-brain-axis.

This Figure illustrates a healthy and senescent gut microbiota and resulting pathological inflammation. By Nagpal et al (2018).

1.1.5 Dysbiosis & disease

There are large variations in the gut microbiota composition between persons and with time. It is therefore difficult to determine what exactly constitutes a healthy microbiome. Nonetheless, it is abundantly clear that 'dysbiosis' of the microbiota is associated with increased risk for disease and frailty (Figure 1.2). Dysbiosis is defined as an alteration of the microbiota composition and is frequently associated as a cause or consequence of a disorder. This is underscored by the fact that infants who are hampered in acquiring their gut microbiota early in life, such as per-term infants in an intensive care unit, have an increased change of developing allergies, infections and Irritable Bowel Syndrome (IBS) later in life (Hickey et al., 2012; Hascoët et al., 2011). Moreover, administration of (bacteria depleting) antibiotics

is associated with increased susceptibility to pathogenic colonization (Sekirov et al., 2008), especially during the first year of life, highlighting the importance and protective role of the endogenous microbiota. While cause and effect relationships frequently remain to be determined, strong associations are reported between an altered gut microbiota composition and intestinal disorders such as: Inflammatory Bowel Disease (IBD), IBS, Celiac Disease, *Clostridium difficile* infection (CDI), and Colorectal Cancer (de Vos & de Vos, 2012). Dysbiosis of the gut microbiota may also manifest as a disorder outside the GIT, as the gut microbiota is reported to affect metabolic, biochemical and neural pathways (Carabotti et al 2015). Multiple studies indicate that dysbiosis of the gut microbiota is associated with mental stress, Alzheimer's disease, Parkinson's disease, Autism Spectrum Disorders, and a host of other indications (de Vos & de Vos, 2012; Rinninella 2019). As an altered gut microbiota composition is associated with illness, it propelled the idea that intervention with external microbes may reduce the risk of such disease.

1.2 PROBIOTICS

1.2.1 Microbial intervention

It was first postulated that interference with microbes could prevent or treat disease in the early 1900s by Élie Metchnikoff (Metchnikoff, 1908). Medical intervention with fermented foods (containing such microbes), however, is ancient practice (Selhub et al 2014). Metchnikoff hypothesized that host-friendly microbes found in fermented milk could replace harmful microbes in our gut, and thereby promote wellbeing and prolong human life. Indeed, it was later demonstrated that consumption of fermented dairy products is associated with improved GIT health and overall wellbeing (Parvez et al 2006). The host-friendly bacteria found in these fermented foods were called probiotics (Lilley and Stillwell, 1965), a term that is still used in contemporary culture. The World Health Organization defines probiotics as: ''live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Morelli & Capurso, 2012).

1.2.2 Probiotic applications

Any type of microorganism could potentially be considered a probiotic. Nonetheless, most probiotic products are developed with bacteria, particularly lactic acid producing bacteria such as bifidobacteria and lactobacilli. These are frequently

used in food fermentation processing and can thus be found in many yogurts, cheeses or other fermented foods. Lactic acid bacteria can also be obtained from plant matter, soil or the GIT of humans and animals (Lee et al., 1999; Fontana et al., 2013). To develop successful probiotic applications, it's generally important that the incorporated bacteria: (1) are neither toxic nor pathogenic, (2) can survive (the acidity and proteolytic activity) of the stomach, (3) are suitable for incorporation in food products or medicine (i.e. able to remain viable after production and processing), and of course (4) confer a health benefit on the host when administered in adequate amounts (Fontana et al., 2013). Although no formal requirements are reported on the minimal 'adequate amount' dosage, it is generally accepted that a probiotic product should contain at least 10^8 Colony Forming Units (CFU) of the bacterial strain.

It is often suggested that probiotics should be able to colonize the GIT in order to convey health benefits on the host. However, many lactic acid bacteria are in fact poor colonizers and tend to have a transient presence that requires continuous consumption of the product (Isolauri et al., 2004). Moreover, while the WHO-definition of a probiotic dictates these microorganisms should be 'live', even dead bacteria may convey health benefits as their membrane structures and cell components interact with the host (Adams, 2010).

1.2.3 Clinical evidence

The clinical applications of probiotics have been studied extensively and increasingly over the past decades (Pandey et al., 2015). Evidence comes from *in vitro*-, animal- and human studies. As the microbiota is involved in numerous systemic pathways and provides the host with a plethora of immunological, metabolic, defensive, neuromodulatory and structural benefits (section 1.1.4), the potential clinical intervention strategies with probiotics are diverse and widespread (van den Nieuwboer, Browne, Claassen., 2016a). Strong associations are found between probiotic intervention and reduced risks for upper respiratory tract infections, Antibiotic Associated Diarrhoea (AAD), infectious diarrhoea and constipation (Kerry et al., 2018; Sánchez et al., 2017). Probiotics also may benefit patients with IBD, IBS, Necrotizing enterocolitis, Obesity, Lactose maldigestion, Atopic Dermatitis, Urinary Tract Infections, and various other indications (Sánchez et al., 2017; Pandey et al., 2015; Marco et al., 2017). In more recent years, the potential role of probiotics has also been studied for the treatment of cancer, carries and a variety of neurological disorders (i.e. Alzheimer, anxiety and depression) (So et al., 2017; Umbrello &

Esposito, 2016; Meurman & Stamatova., 2018). While the therapeutic potential of probiotics is clearly demonstrated by the combined evidence of these studies, compelling evidence is often still warranted per indication (van den Nieuwboer et al., 2016a). Moreover, the effects of probiotics tend to be strain-specific, and the clinical outcomes can thus not be transposed to all probiotics in general but require validation on a strain- or formulation-specific basis (McFarland, Evans & Goldstein., 2018).

1.2.4 Mechanism of action

According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), some mechanisms are uncommon among different strains, but others are widespread among strains of the same species (Hill et al., 2014). Individual strains may have multiple mechanisms of action but a comprehensive understanding of these is often lacking (Lebeer et al., 2018). Nonetheless, the following mechanisms of action are frequently ascribed to probiotics (a schematic overview is provided in Figure 1.3):

- Producing metabolites such as short-chain fatty acids and histamine that may improve gut integrity, alter cell proliferation, and increase anti-inflammatory, antitumorigenic, and antimicrobial functioning (Macfarlane & Macfarlane., 2003; Sartor., 2008; Byrne et al., 2015; Tan et al., 2014; Gao et al., 2017; Sanders et al., 2018)
- Enhancing epithelial barrier integrity by stimulating the epithelial mucosal layer, secretory IgA, antimicrobial peptides and the epithelial junction adhesion complex (Anderson et al., 2010; Zyrek et al., 2007; Stetinova et al 2010; Hooper & Stappenbeck., 2003; Otte & Podolsky., 2004; Rao & Samak., 2013)
- Adhering to the mucosal layer and epithelium lining and thereby competitively inhibiting pathogen adhesion and growth (Bermudez-Brito et al., 2012; Hirano et al., 2003)
- Modulating the composition of the host microbiota through adherence and colonization (Motherway et al., 2011; Hemarajata & Versalovic., 2013)
- Inhibiting pathogen virulence gene and protein expression (Corr et al., 2009)
- Producing organic acids such as lactic acid and acetic acid which have a strong inhibitory effect against Gram-negative bacteria (Alakomi et al., 2000; De Keersmaecker et al., 2006; Makras et al., 2006)
- Producing bacteriocins that act against closely related bacteria or food-borne pathogens (Corr et al., 2009; Spinler et al., 2017)

- Modulating intestinal and systemic immunity and alter the responsiveness of the intestinal epithelia and immune cells (Yan & Polk., 2011; Thomas and Versalovic, 2010; Bron et al. 2011).
- Altering central nervous system signalling through the Gut-Brain-Axis and the hypothalamic-pituitary-adrenal (HPA) axis (Wang et al., 2016; Bercik et al., 2011; Bravo et al., 2011)
- Modulating gene expression in host tissues at distance from the gastrointestinal tract, such as the liver, by influencing the gene expression of mucins, Toll-like receptors, caspases, nuclear factor-κB, and interleukins (Plaza-Diaz et al 2014; D'argenio et al 2013)
- Producing and supplying vitamins such as vitamin K and water-soluble B vitamins (Gu & Li., 2016; Cani 2018)
- Influencing levels of hormones such as Ghrelin, Leptin, adipsin and adiponectin (Clarke et al., 2014; Kadooka et al., 2010; Mallappa et al., 2012; Ohlson et al., 2008; Mencarelli et al., 2011)
- Synthesising enzymes such as lactase to promote lactose digestion in the small intestine (de Vrese *et al.,* 2001)

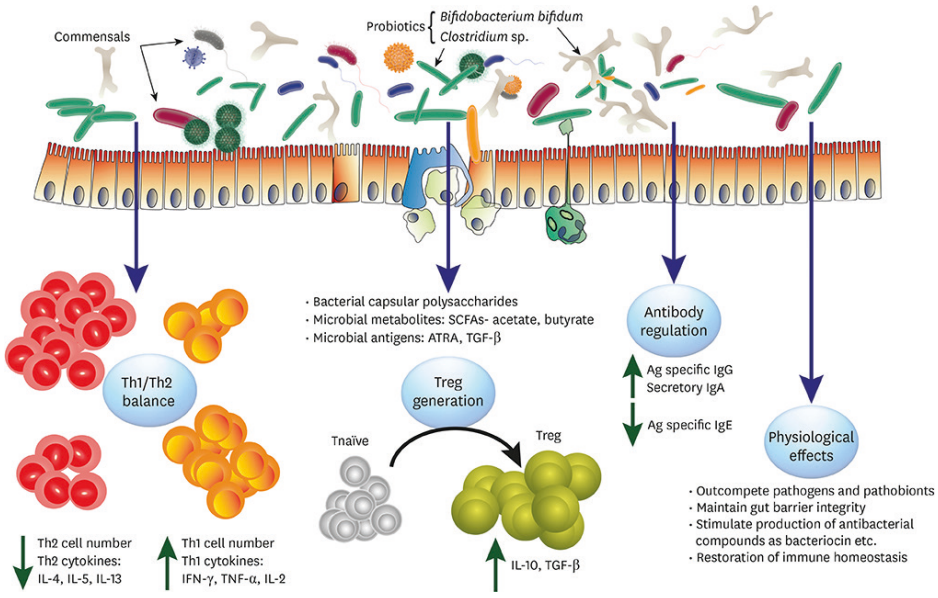


Figure 1.3 Known mechanisms whereby probiotics impact the gut microbiota.

This Figure illustrates known mechanisms by which probiotic bacteria may impact on the gut microbiota. By Sharma & Im (2018).

1.2.5 Prebiotics & synbiotics

The effectiveness of probiotics may be enhanced by supplementing the formulation with prebiotics such as Fructooligosaccharide (FOS) and Galactooligosaccharide (GOS). These carbohydrates provide probiotic bacteria with sustenance that may improve their viability. A combination of probiotics with prebiotics is called a synbiotic formulation. Prebiotics can also be administered without probiotics and may benefit the host by providing nourishment to endogenous gut microbes that enables their sustained growth (De Vrese, & Schrezenmeir., 2008; Pandey et al., 2015).

1.2.6 Safety

While there has been substantial debate on the safety of probiotics in the past (Morrow et al., 2012), recent publications clearly indicate that orally consumed probiotics have an excellent safety profile with few reported adverse events (Cabana et al., 2019; Didari et al., 2014). Multiple meta-analyses demonstrate that the consumption of probiotics is safe, even for young children, elderly and immunocompromised patients (Van den Nieuwboer et al., 2014a; Van den Nieuwboer et al., 2014b; Van den Nieuwboer et al., 2015; Larsen et al.,2017). Commonly used lactic acid bacteria are therefore Generally Recognized as Safe (GRAS) for human consumption and have received such, or similar, clearance from regulatory authorities globally (Brodman et al., 2017; Elshaghabee et al., 2017). Nonetheless, the introduction of a novel microorganism without such clearance warrants thorough safety assessments prior to market authorization (Brodman et al., 2017).

1.3 PROBIOTIC MARKET

1.3.1 Products & markets

One of the first commercial probiotic products originated in Japan in 1935. Here, Dr. Minoru Shirota isolated and cultured a lactobacillus strain (*L. casei* Shirota) and used it to produce a fermented probiotic dairy drink called Yakult (Yakult Europe, 2019). The popular drink is still commonly sold and consumed globally to date but has gained tremendous competition as many other probiotic products have flooded the commercial market. These exist in all shapes and forms and are created for both humans and animals (Wang et al., 2016). Typically, we distinguish two types of

commercial products for human consumption: (1) probiotic foods and beverages, such as yoghurts, chocolates and fermented dairy drinks, supplemented with one or multiple probiotic strains (>10^8 CFU) and (2) probiotic dietary supplements, such as capsules, powders or suppositories, which usually contain a variety probiotics species/strains that are selected based on their viability and potential to prevent a specific disease. However, an increasing number of unsubstantiated products are also reaching the market, such as probiotic shampoos, deodorants and matrasses (Sanders., 2008). The scientific rationale and clinical evidence behind these products are usually marginal. Combined, the probiotic market was estimated at 49 billion dollars in 2018 and is expected to reach 69 billion dollars by 2023 (MarketsandMarkets, 2018; Caselli et al., 2013; Grand View Research, 2016). Moreover, a strong increase is seen in the number of probiotic patent applications that are filed over the past decades, indicative of long-term investment strategies and trust in continued growth of the market (Dixit et al., 2016).

1.3.2 Regulations & health claims

The regulatory landscape for probiotics is diverse and ambiguous. While many probiotics were initially sold as medical devices, primarily due to the relatively low barriers for approval by regulatory bodies, recent policy changes explicitly state that probiotics are no longer recognized as a medical device (EU Directive 2017/745). Many companies therefore need to reclassify their existing products and are looking to develop alternative marketing strategies. Probiotics can also be sold as a medicine, by filing a drug application to organizations like the European Medicine Association (EMA) or the United States' Food and Drug Administration (FDA) (Van Norman, 2016). These products should then treat, cure or prevent disease in a patient population. Obtaining market authorization for a novel medicine, however, is a costly and lengthy undertaking with enormous monetary investments in clinical trials (Morgan et al., 2011). Most probiotics are therefore sold as nutritional/dietary supplements, and thus are amenable to regulations by food authorities like the European Food and Safety Authority (EFSA). Dietary supplements can be sold on the open market in Europe if they have a Qualified Presumption of Safety (QPS), comparable to the GRAS status in the United States, but to publicly market that a product has specific health promoting properties (i.e. on the product's packaging), health claim approval needs to be granted first (EFSA, 2016). For such a claim, the EFSA states that a relationship between a specific food and maintenance of good health needs to be established, or a relationship between the food and reduced risk for the disease. The focus is here on healthy populations as opposed

to patients, and the claim should be substantiated with demonstratable changes in generally accepted biomarkers reflecting the risk of disease (EFSA, 2016). While extensive research has been performed with probiotics, most clinical trials have been conducted in patients or subjects at risk of a specific disease rather than a healthy population, complicating the health claim approval process for nutritional supplements (Gibson et al., 2011). Moreover, it is reported a substantial number of clinical trials with a probiotic intervention lack sufficient power, appropriate randomization and blinding, thereby diminishing the weight of the reported evidence (van den Nieuwboer et al, 2016b). To date, no probiotic health claim has been approved by the EFSA and perceived health benefits can thus not be communicated to consumers (Dronkers et al., 2018; Turck et al., 2017; Bröring et al., 2018). Consumers are hence faced with Latin terms on the product's labelling (i.e. *Lactobacillus rhamnosus* ATCC 53103) as opposed to the intended health indication. As these are not easily understood they create confusion rather than clarity and hamper the probiotic innovation process.

1.4 INNOVATION PROCESS

1.4.1 Defining innovation

The concept of innovation is complex and polysemous, as there are many academic definitions which vary according to their context (e.g. firm, society or individual) and theoretical background. Baregeh et al (2009) defines innovation as 'a multi-stage process whereby organizations transform ideas into new and/or improved products, services or processes, in order to advance, compete and differentiate themselves successfully in the marketplace'. This view clearly highlights that the socioeconomic benefits of an organization may drive innovation. However, the implications of these improved products or services may reach well outside the scope of a single firm or marketplace. Innovation in healthcare, for instance, has the potential to drive change and redefine healthcare's economic and social potential (Weberg et al., 2009). We therefore define probiotic innovation in the present study as: a multi-stage process whereby organizations transform ideas into new or improved products, in order to differentiate themselves successfully in the marketplace and redefine the socioeconomic potential of healthcare.

1.4.2 Microbiota Valorisation & Tech Transfer Cycle

Innovation models can be used as a tool to study valorisation barriers and provide a frame of reference for identifying and advancing change ideas that are most likely to generate value for sustained growth. Innovation systems were initially described as linear or multi-step processes characterized by successive development phases that aim to bridge applied research and socioeconomic benefits (Godin, 2006). These models, however, have been criticised for their perceived one-directionality and lack of iterations (Berkhout et al., 2006). Cyclic innovation models were proposed that take into consideration the multiple feedback loops between industry segments and consortium partners (Berkhout et al., 2010). This concept is used by van den Nieuwboer and colleagues (2016b) to study probiotic innovation barriers, by adapting the Valorisation & Tech Transfer Cycle (Pronker et al., 2013) for research and development on the human microbiota (Figure 1.4). This model distinguishes 3 interrelated segments:

- **The Scientific Discourse**: where the proof of principle of an initial idea is evaluated through empirical research before realizing it through business formation and intellectual property protection.
- **The Development Discourse**: where the proof of concept, safety and efficacy of a product are established through (pre)clinical research trials, before scaling up the product for market introduction.
- **The Market & Society Discourse**: where consumer feedback and unmet medical need articulation feed back into fundamental research and ideation.

We use the Microbiota Valorization & Tech Transfer Cycle (CVM) of van den Nieuwboer and colleagues (2016b) as our conceptual model to study key drivers of the probiotic innovation process and their interrelationship.

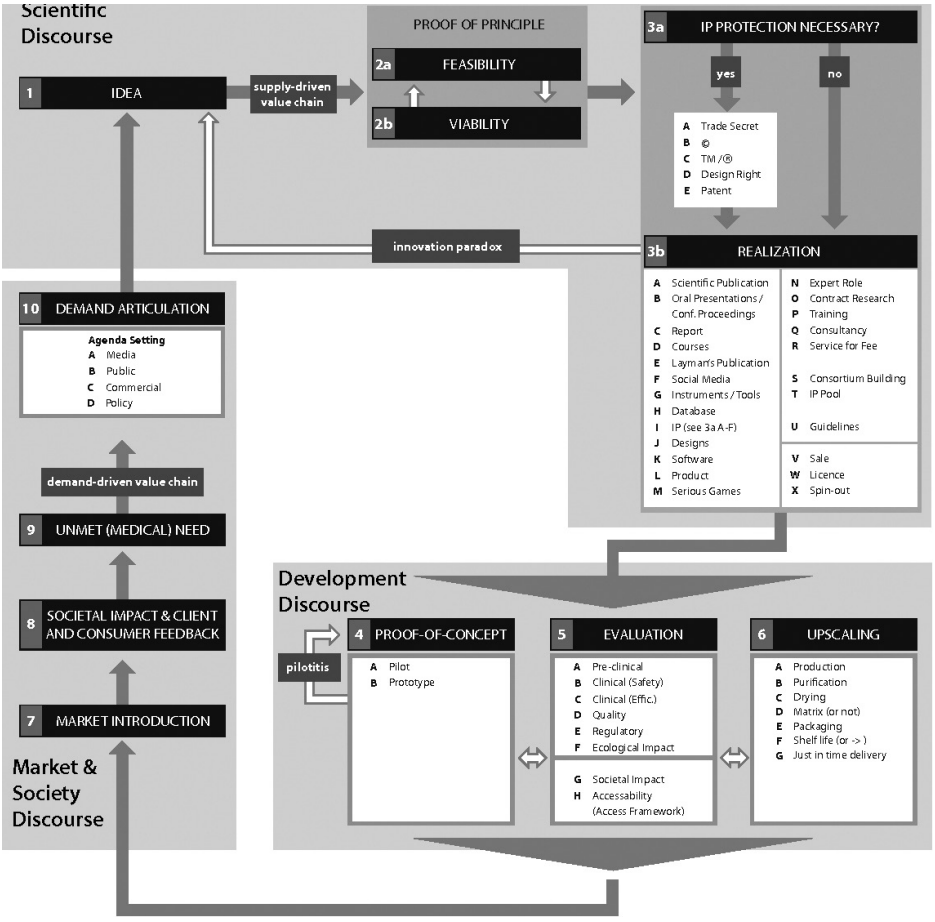
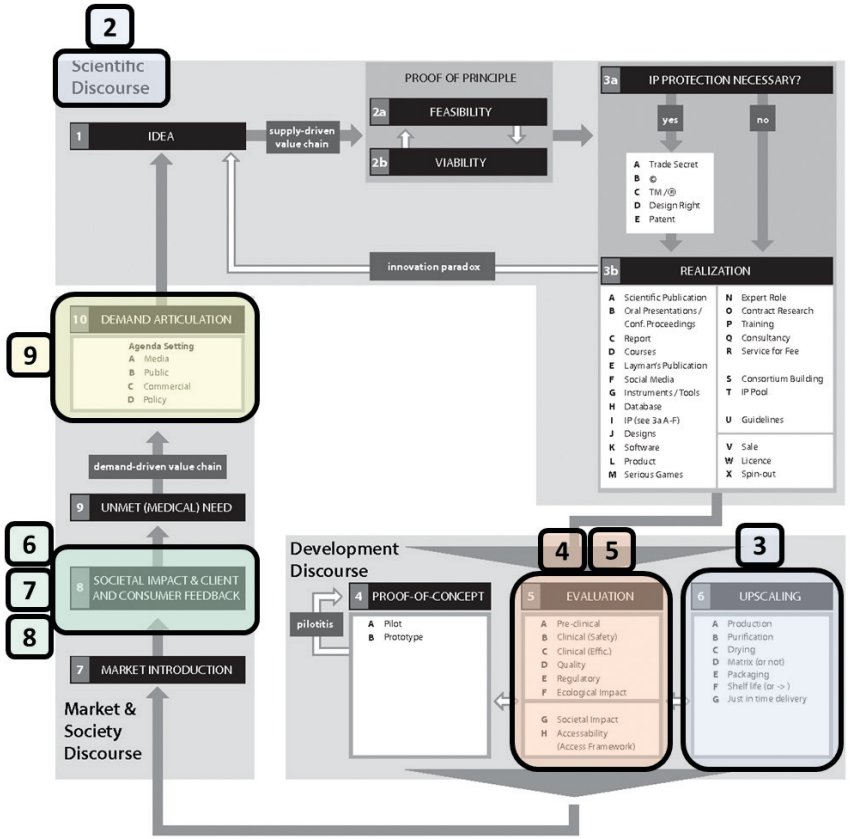


Figure 1.4 The Microbiota Valorisation & Technology Transfer Cycle.

This Figure portrays the three interrelated discourses and corresponding steps of the cyclic innovation model used to study innovation barriers in microbiota R&D. By van den Nieuwboer et al (2016b).

1.5 PROBLEM STATEMENT & RESEARCH DESIGN

While the probiotic industry has shown tremendous growth over the past decades, the innovation process for probiotics is hampered considerably according to Key Opinion Leaders. Probiotics are not consistently used in clinical practice, no European health claim has been approved to date and there remains a lack of fundamental knowledge on probiotics and their interaction with the host (van den Nieuwboer et al., 2016b).



Chapter 2 & 3

Which critical challenges do innovators face when developing probiotic applications?

Chapter 4 & 5

What are the barriers and opportunities for bowel habit improvement in nursing homes with probiotic intervention?

Chapter 6, 7 & 8

What are the perceptions of physicians and patients towards probiotics?

Chapter 9

How should research be prioritized for health claim approval in the adult population?

Figure 1.5 Research Chapters per Innovation Domain.
This Figure portrays the chapters of this thesis, divided over the different domains of the CVM.

To cultivate the therapeutic and socioeconomic benefits of probiotics for consumers and patients, and to stimulate growth of the probiotic market, it is vital that these barriers are addressed and abated, and that new ones are continuously researched.

This thesis therefore sets out to study key barriers to the probiotic innovation process to advance research & development on live microorganisms for the promotion of human health.

To attain this objective, a mix methods approach is adopted using a combination of literature studies, quantitative surveys, systematic reviews, meta-analyses, health economic models and in-depth interviews. The CVM is used as a frame of reference on probiotic innovation. For each discourse, prominent barriers to innovation are reviewed and corresponding research objectives formulated (Fig 1.5). An overview of all research objectives, study methods and corresponding chapters of this thesis are described below and in table 1.1.

1.5.1 Which critical challenges do innovators face when developing probiotic applications?

Innovators are faced with several persistent challenges throughout the production and development of probiotic applications (Jankovic et al., 2010; van den Nieuwboer et al., 2016b). As our first objective, we therefore aim to identify critical barriers associated with the access to-, research on- and upscaling of probiotic microorganisms (Fig 1.5 Scientific discourse & Upscaling).

In Chapter 2, we explore from a regulatory perspective how the Nagoya Protocol on Access to Genetic Resources restricts research & development on probiotic microorganisms. We review the regulatory framework of the Nagoya Protocol and the barriers associated with the access and utilization lactic acid bacteria for the development of probiotic applications. A literature study is conducted that analyses existing guidance documents on compliance with Nagoya Protocol, and subsequently, a decision framework was developed to guide probiotic innovators.

In Chapter 3, we assess the development risks during production and packaging that may alter the quality of a probiotic product. The most substantiated carrier matrices, factors that influence probiotic functionality during upscaling, and matrix effects on shelf-life, gastrointestinal tract survival and clinical efficacy are reviewed.

1.5.2 What are the barriers and opportunities for bowel habit improvement in nursing homes with probiotic intervention?

Probiotics are not consistently used in clinical practice, despite their apparent clinical potential and increasing prescription rates among medical doctors (van den Nieuwboer et al., 2016b; Ababneh et al., 2019; Browne et al., 2019). This limited use could be ascribed to a lack of safety, efficacy or accessibility of the intervention, as those factors are vital for the success of a probiotic application (Fig 1.5 Evaluation). To advance innovation within Evaluation domain, we therefore aim to determine this potential of probiotics in a population of nursing home residents with regard to bowel habit improvement.

In Chapter 4, we conduct a literature review on studies reporting the effects of probiotic intervention in institutionalized elderly to evaluate the opportunities for bowel habit improvement in nursing homes. Here, we focus on probiotic safety and efficacy. Subsequently, the health economic potential of probiotics in institutionalized elderly with chronic constipation is assessed in Chapter 5 to determine the accessibility/affordability of probiotics for this patient population. To attain this objective, we conduct a meta-analysis of clinical research trials and performed a quantitative survey with nursing home employees (N = 118).

1.5.3 What are the perceptions of physicians and patients towards probiotics?

Negative perceptions and low acceptance among physicians and consumers are key barriers to probiotic innovation, according to van den Nieuwboer and colleagues (2016b). It is crucial to obtain such consumer feedback on the quality and impact of a product after market introduction so that it may be improved for future reference (Fig 1.5 Societal impact & client and consumer feedback). Here, we therefore seek to explore the perceptions of both physicians and patients towards probiotics.

In Chapter 6, a post-marketing study with qualitative interviews is performed to evaluate the attitudes of 23 ulcerative colitis patients towards probiotics and assess the impact of supplementation on their quality of life. The perceptions on probiotics and prescription rates of medical doctors (MD) are subsequently evaluated in Chapter 7 based on a quantitative survey with 415 Dutch MDs. In Chapter 8, a

follow-up survey is performed to evaluate the attitudes of 1318 General Practitioners from eight European countries towards probiotics.

1.5.4 How should research be prioritized for health claim approval in the adult population?

No probiotic health claim has been approved in Europe to date, despite the increasing amount of clinical trials that are being performed with probiotics. Unable to communicate the intended health effects to consumers, this forms a prominent barrier to innovation (de Simone., 2018), which can (in part) be attributed to the wide range of potential therapeutic applications and a diluted distribution of research efforts. Here, we therefore aim to review the current clinical evidence of two of the best documented probiotic strains (*Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12) to prioritize future research for health claim approval (Fig 1.5 Demand Articulation).

In Chapter 9, we review 92 clinical trials that have been performed with LGG and BB-12 over thirteen different health domains. Research priorities for health claim approval are subsequently formulated based on 42 studies that have been performed in healthy adults or patient populations that are considered representative for effects in the general population.

Table 1.1 Overview of chapters

Chapter:	Title:	Method:	CVM Domain:	Reference:
2	The Nagoya Protocol on Access to Genetic Resources and Benefit Sharing: best practices for users of Lactic Acid Bacteria	Literature review and guidance documentation	Scientific Discourse	Flach et al., 2019
3	The underexposed role of food matrices in probiotic products: Reviewing the relationship between carrier matrices and product parameters.	Systematic Review	Upscaling	Flach et al., 2018c
4	Probiotics for healthy ageing: Innovation barriers and opportunities for bowel habit improvement in nursing homes	Literature Review	Evaluation: *Societal Impact*	Larsen et al., 2017

Table 1.1 Continued

Chapter	Title:	Method:	CVM Domain:	Reference:
5	Economic potential of probiotic supplementation in institutionalized elderly with chronic constipation	Meta Analyses, Health Economic Model & Quantitative Survey	Evaluation: *Accessibility*	Flach et al., 2018a
6	Probiotics for improving quality of life in ulcerative colitis: Exploring the patient perspective	Post-marketing study with 23 Ulcerative Colitis patients	Client and Consumer Feedback	van der Waal et al., 2019
7	Medical doctors' perceptions on probiotics: Lack of efficacy data hampers innovation	Quantitative survey with 415 Dutch Medical Doctors	Client and Consumer Feedback	Flach et al., 2017
8	European General Practitioners perceptions on probiotics: Results of a multinational survey	Quantitative survey with 1318 European General Practitioners	Client and Consumer Feedback	van der Geest et al., 2019
9	Probiotic research priorities for the healthy adult population: A review on the health benefits of Lactobacillus rhamnosus GG and Bifidobacterium animalis subspecies lactis BB-12	Systematic Review	Demand Articulation	Flach et al., 2018b

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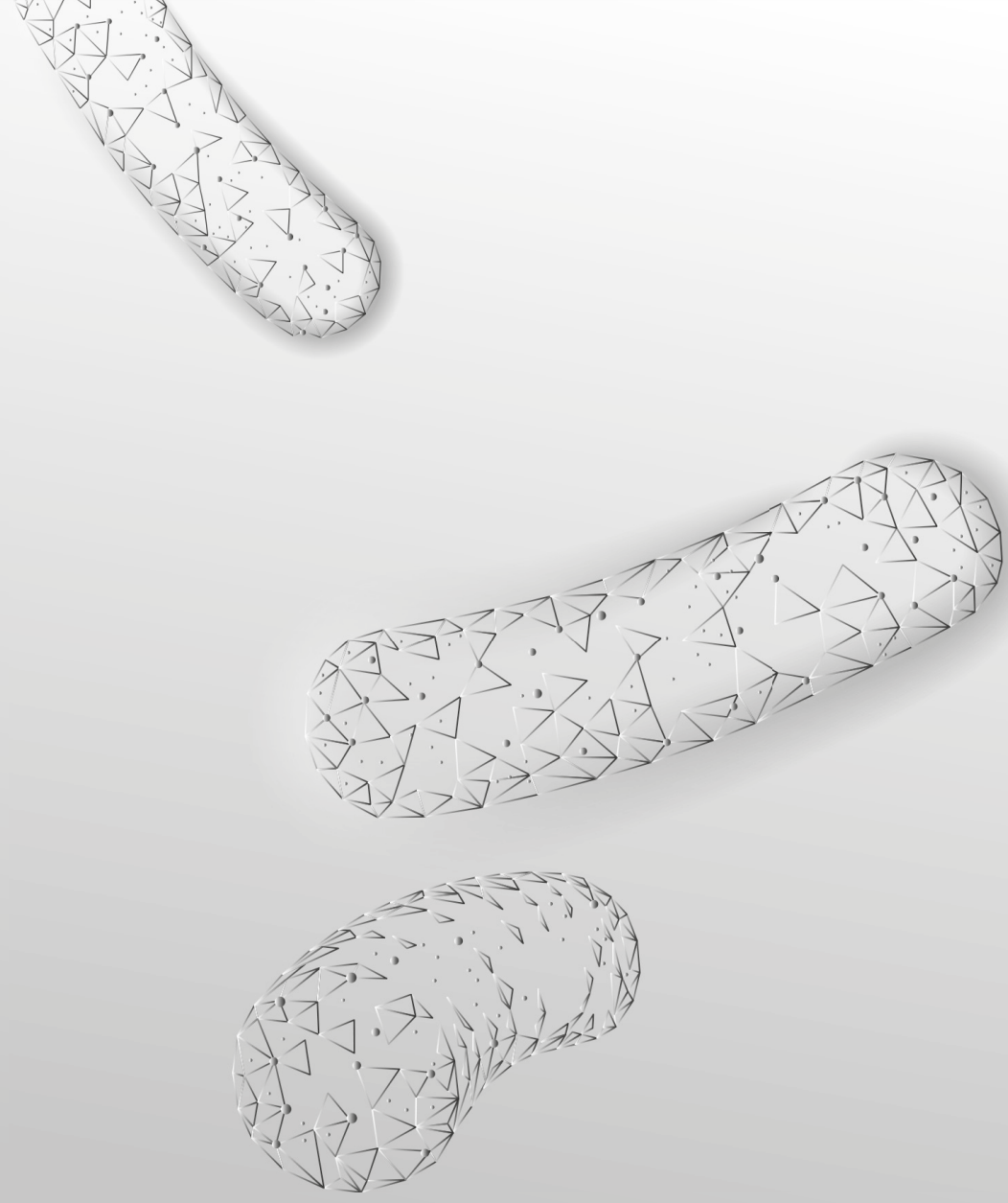
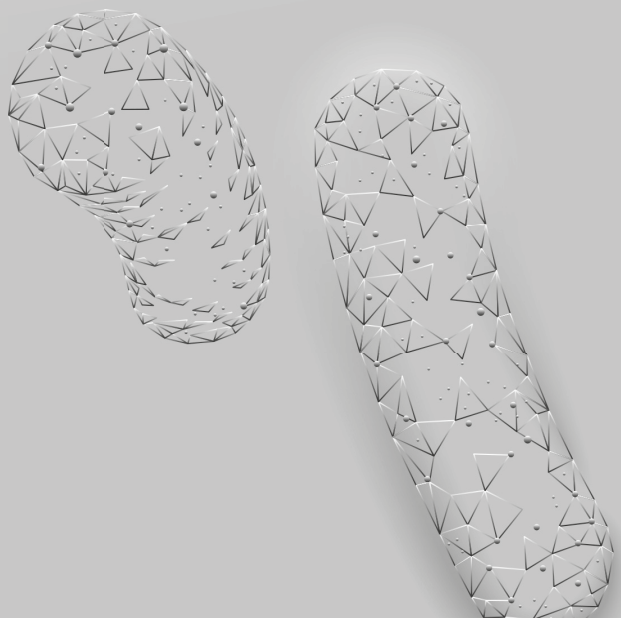


CHAPTER 10.

Discussion & Conclusion



10.1 OUTLINE

Medical intervention with probiotics has the ability to drive change and redefine healthcare's socioeconomic potential. However, the valorisation cycle for probiotics appears to be hampered and currently prevents rapid progress (van den Nieuwboer et al., 2016b). This thesis therefore sets out to study critical barriers to the probiotic innovation process to advance research & development on live microorganisms for the promotion of human health. The CVM is used as a frame of reference on probiotic valorisation to study persistent challenges and key innovation drivers (Section 1.4.2). The following research objectives were formulated and are addressed in the individual chapters of this thesis:

1. Which critical challenges do innovators face when developing probiotic applications?
2. What are the barriers and opportunities for bowel habit improvement in nursing homes with probiotic intervention?
3. What are the perceptions of patients and physicians towards probiotics?
4. How should research be prioritized for health claim approval in the adult population?

Here, we present our key findings, their implications and recommendations for future research. The chapter ends with a general conclusion and a discussion on the validity of the utilized research methodologies.

10.2 CRITICAL CHALLENGES IN THE DEVELOPMENT OF PROBIOTIC APPLICATIONS

Developing successful probiotic applications can be a lengthy and complex undertaking. Throughout the production and development process, probiotic innovators are faced with several persistent challenges (Jankovic et al., 2010; van den Nieuwboer et al., 2016b). Here, we explored how Access and Benefit Sharing legislation restricts research and development on probiotic microorganisms (Chapter 2), and how production, processing and packaging may alter the quality and functionality of a probiotic product (Chapter 3).

10.2.1 Key findings: Access to genetic resources

Probiotics are genetic resources that are amenable to Access and Benefit Sharing legislation and the relevant provisions of the Nagoya Protocol (CBD, 2018). To conduct research and development on these microorganisms, innovators therefore need to obtain prior informed consent from the provider country of the material and negotiate mutual agreed terms for their use. The objective of the Nagoya Protocol is to promote transparency on the management of genetic resources through fair and equitable sharing of benefits arising from their utilization, thereby contributing to the conservation of biological diversity and the sustainable use of its components globally (CBD, 2010). However, we demonstrate in Chapter 2 that the legal requirements to access and utilize probiotics microorganisms from countries that ratified the Protocol can be disproportionally high or unattainable. Critical barriers include: (1) the decentralized nature of the Nagoya Protocol which gives rise to a high diversity of local regulations, (2) the inability to trace genetic resources from widely available commodities back to a single country of origin, and (3) the equivocality of the Protocol's scope, for instance, regarding the inclusion of genetic sequence data and genetic resources from the human microbiome.

While Access and Benefit Sharing legislation is vital to negate biopiracy and ensure fair sharing of benefits (Buck & Hamilton., 2011), the limitations associated with the default implementation of the Nagoya Protocol can make compliance for users a rather daunting task. It is feared that probiotic innovators are tempted to source their genetic resources from countries that choose not to exert their sovereignty rights, thereby leaving many indigenous (and potentially beneficial) probiotic strains underutilized. This directly goes against the objective of the Nagoya Protocol and restricts the flow of novel probiotic species unto the market.

10.2.2 Key findings: Production & packaging

Probiotics are also live microorganisms that are affected by their surroundings. We demonstrate in Chapter 3, that a host of external factors can alter probiotic viability during production, processing and packaging. The most critical factors include: (1) food ingredients and additives, (2) temperature, (3) pH, (4) water activity, (5) oxygen contents or redox potentials, (6) packaging aspects and (7) competing bacteria. For instance, high oxygen contents and low pH are generally correlated negatively with probiotic viability, although certain species and strains are better equipped to deal with such environments. These factors can also influence the geno- and phenotypes of cells in response to their environment, thereby potentially altering the probiotic effect (Bisanz et al., 2014; Reid, 2015). It is thus crucial to carefully monitor probiotic stability during production and processing, and for the same reason, to choose the right carrier matrix for administration. Probiotics can be administered in a plethora of different matrices, including yogurts, dairy drinks, fruit juices, chocolates, ice-cream and (lyophilized) powders or capsules, each presenting their own benefits and trade-offs. In simulated gastric conditions, for instance, dairy- and water-based products seem to outperform freeze-dried capsules on probiotic survival (Fredua-Agyeman & Gaisford, 2015). *In vivo* studies furthermore reveal strain-dependent matrix effects on GIT survival and probiotic stability. For instance, (1) faecal excretion levels of *L. salivarius* UCC118 were found on average to be 15 times higher in fresh milk than in fermented milk (Collins et al., 2002) and (2) probiotic cell counts were found to decrease with 1-2 log CFU/ml in fruit juice and with less than 1 log CFU/ml in pasteurized milk after two weeks of storage (Saarela et al.,2006). However, the amount of clinical studies evaluating two or more matrices in their potential as optimal carrier for probiotic administration is scarce.

We show here the external environments of probiotics can alter their functionality throughout the entire development cycle. Whereas lowered viability may reduce clinical efficacy, changes in the geno- and phenotypes can alter the host response and introduce potential safety concerns for consumers (Sanders et al., 2014). There is thus a clear need to (re)identify the genetic and phenotypic differences between reference- and product strains after production and processing. These findings are underlined by a recent study by Ansari and colleagues (2019) which revealed that the contents of many probiotic products do not match the reported strains on their packaging and that certain strains have altered gene expressions when compared with the native strain.

10.2.3 Recommendations

Current research on probiotics seems to exhibit a disproportioned distribution of efforts, where most scientific studies have evaluated the strain-specific clinical effects of probiotics, but the effects of carrier matrices and production processes remain largely underexposed. Consequently, there is a lack of fundamental knowledge on their effects on cell proliferation, gene expression and probiotic working mechanisms (Reid., 2016; van den Nieuwboer et al., 2016b), which calls for additional studies on the influence of production environments and carrier matrices. For novel microbial species, however, such research and information exchange is currently curtailed under the Nagoya Protocol. We therefore recommend that a multilateral system (MLS) and associated treaty are established in which conditions for access and use of lactic acid bacteria are agreed between all members and translated in standardized Material Transfer Agreements (Reichman et al., 2015; Ribeiro et al., 2018). Ratifying countries thereby agree to make their genetic diversity and associated knowledge available to all through the MLS, where contracting Parties share a set of standardized rules of facilitated access. This system reduces costly and time-consuming efforts of users to negotiate contracts with individual parties or countries, thereby further stimulating innovation.

10.3 BARRIERS AND OPPORTUNITIES FOR BOWEL HABIT IMPROVEMENT IN NURSING HOMES

The socioeconomic success of a probiotic application is dependent on its impact on society as well as the accessibility for the intended population (van den Nieuwboer et al., 2016b). Probiotics therefore need to be safe, effective and affordable. Here, we explored the potential of probiotics for bowel habit improvement in nursing home care to advance innovation within this domain, by reviewing the safety, efficacy, and financial impact of probiotic supplementation (Chapter 4 and 5).

10.3.1 Key findings

Chronic constipation and diarrhoea are common GIT disorders in institutionalized elderly that are associated with age-, diet- and polypharmacy-related microbiota perturbations (Ticinesi et al., 2017; Odamaki et al., 2016). Both indications have a severe impact on health-related quality of life and carry a substantial economic burden (Bongers et al., 2009; Frank et al., 2002). The unmet health need seems

to be highest with respect to constipation, as we demonstrate in Chapter 4 & 5 that approximately half of nursing home residents are constipated on average compared with a median of 16% in the general population. The prevalence of diarrhoea in nursing homes is lower, albeit still high, with a reported average of 13%. Probiotics can be effective in improving the bowel habits of elderly residents, as indicated by multiple clinical studies with a variety of probiotic strains (Chapter 4). In chapter 5, we conducted a meta-analysis on studies reporting the defecation frequency and stool consistency of institutionalized elderly with constipation before and after probiotic supplementation, to show that the intervention may reduce the prevalence of constipation by 28%. We also demonstrate that probiotic supplementation can reduce the conventional treatment costs for constipation management in these institutions as probiotics are relatively affordable and can be easily implemented (i.e. replacing regular milk with a probiotic version). An average sized nursing home with 100 residents and a constipation prevalence of 42%, may therefore save between €8,000–€25,000 (9–28%) annually in constipation-related expenses when supplementing all residents with probiotics. In line with previous safety studies (Cabana et al., 2019; Van den Nieuwboer et al., 2014a) we also confirm that probiotics are safe for consumption in this population, as no significant differences were observed on the total number of adverse events between probiotic- and control groups (apart from a higher incidence in flatulence (Chapter 4)).

There is a clear unmet health need within nursing home care to improve quality of life by reducing (co-) morbidity and lowering associated health care costs. This need is underlined by a strikingly high prevalence of constipation in nursing homes and an unprecedent rate of aging in our society (WHO, 2011). It is projected that over 20% of the world population will be 60 years or older in 2050, compared with 8% in 1950. This calls for novel intervention strategies in nursing home care that are safe, effective and economically viable. We demonstrate that probiotics have this potential for constipation management, yet their usage remains limited within medical communities and nursing home care. Low prescription rates are often attributed to a lack of quality controlled clinical trials (van den Nieuwboer et al., 2016b), and indeed the clinical studies we reviewed here present several methodological limitations. First off, the sample sizes of these studies are relatively small (or experienced large drop-out rates), with only a single study including more than 50 participants in the per-protocol-set (Pitkälä et al 2007). Most studies were also either uncontrolled, open-label or adopted relatively short intervention periods, and as each study utilized different probiotic strains and carrier matrices, the combined data is highly heterogenous and warrants further clinical evaluation.

10.3.2 Recommendations

Compelling and high-powered clinical trials are needed to foster innovation and convince elderly care physicians, policy makers, and food or drug administrations of the added benefits of probiotic supplementation in this population (Gibson et al., 2011; Flach et al., 2017). Increasing cooperation is therefore needed between researchers, nursing homes and ethics- & regulatory committees, as adversities inherent to clinical research in nursing homes currently prevent rapid progress: i.e. obtaining informed consent from incapacitated elderly, involving staff members and obtaining regulatory and ethical approval. To further substantiate the health economic potential, we recommended that the impact of probiotic intervention on the workload for nursing home employees is addressed in these future studies, as this has not been previously evaluated (but makes up 80% of the conventional treatment costs for constipation care). Similarly, the effects of probiotic intervention on the laxative use by nursing home residents generally appear to be underreported, with little insight given into the prescription policies of the institutions (e.g. evaluation periods and constipation criteria) and the course of treatment over time. Finally, our health economic calculation only considers the costs associated with constipation management, whereas probiotic supplementation may benefit the host in various other ways. For example, probiotic supplementation may reduce (antibiotic associated) diarrhoea in elderly nursing home residents (Rondanelli et al., 2015; Hamilton-Miller, 2004) and could potentially reduce treatment expenditures in this area as well, which calls for additional health economic evaluations and a consolidating approach.

10.4 PERCEPTIONS OF PHYSICIANS AND PATIENTS TOWARDS PROBIOTICS

Negative perceptions and low acceptance among physicians and patients are reported as key barriers to the probiotic innovation process (van den Nieuwboer et al., 2016b). Here, we sought to explore the perceptions of both users and prescribers of probiotics and analysed their underlying cause. In chapter 6, we reviewed the perceptions of Ulcerative Colitis (UC) patients, and in chapter 7 & 8, we explored the attitudes of Medical Doctors (MDs) and General Practitioners (GPs) towards probiotics.

10.4.1 Key findings

UC is the most common form of IBD, characterized by mucosal inflammation and ulcers on the inner lining of the human colon and rectum (Conrad et al., 2014). In Chapter 6, we show that probiotic supplementation may improve QoL in this population, as 64% of UC patients who had consumed a probiotic formulation on a regular basis ('users') reported beneficial effects ('responders'). Probiotic effects in the physical domain were most prominent, with half of responders experiencing a decreased stool frequency and enhanced stool texture. The vast majority (88%) of responders also deemed the observed effects to be relevant or even highly relevant in terms of improving their QoL, whereas none of the users reported negative effects of consumption. All 23 interview participants expressed a positive general attitude towards probiotics, frequently reported by users as a curiosity towards the potential beneficial effects on their QoL (by 64% of users, 0% of non-users; 39% of total) and as a belief in the underlying theoretical rationale (21% of users; 44% of non-users; 30% of total). However, 44% of non-users and 7% of users (22% of total) reported to be positive yet cautious, awaiting convincing evidence of beneficial effects. Similar results are observed for prescribers of probiotics in Chapter 7 & 8. Here we demonstrate that between 50-80% of MDs and GPs in Europe (N = 415 and N = 1318, respectively) prescribe probiotics in their practice at least sometimes ('Advisors'), primarily for AAD, Infectious Diarrhoea, Abdominal discomfort, IBS and IBD. While half of MDs and GPs indicate that they perceive probiotics to be safe and that there is sufficient clinical evidence regarding the efficacy of probiotics, there is a clear need for further clinical evaluation as the primary reason not to advice probiotics was a lack of evidence regarding efficacy (53%). This was also the preferred type of future information for most MDs and GPs.

Distal factors (such as the characteristics of an innovation) determine consumers' intention to accept an innovation through proximal factors (e.g. social norms) (Ronteltap et al., 2007). In this regard, the doctor-patient relationship is an important driver of consumer acceptance and steers public opinion (Robinson et al., 2004; Noble., 2016). While many physicians prescribe probiotics in their practice (at least sometimes), 40-50% still indicated there is insufficient evidence regarding probiotic safety or efficacy. Similarly, patient communities expressly state that they are waiting for more convincing evidence of beneficial effects. To stimulate probiotic innovation by improving the perceptions of patients and physicians towards probiotics, more compelling and controlled clinical trials are therefore required.

10.4.2 Recommendations

While the need for further clinical substantiation is evident and often reiterated, there are other factors that drive prescription behaviour and consumer acceptance. In Chapter 6, we show that conventional media (such as TV and Radio) are associated with negative perceptions and lowered prescription rates among physicians. Other studies report that factors such as social team dynamics, hierarchy, time pressure, personal norms, prior experiences, culture and religion are all factors that influence the prescription behaviour as well (Warremana et al., 2018). The fact that probiotics are frequently not adopted in guidelines for physicians (and prescription would therefore go against the social/cultural norm), may be another prominent barrier to innovation (NHG., 2019; Randel., 2018). Further research is therefore needed to indicate whether, and to what extent, these and other factors are of influence on the perceptions of physicians and their prescription behaviour to advance innovation in this domain. Moreover, the list of indications for which probiotics might be beneficial is long and expanding (Foligne et al., 2013). As most clinical effects of probiotic are strain-specific and cannot be extrapolated to other species, a physician needs to prescribe different bacterial strains for different indications (McFarland., 2018). It appears that GPs are provided with insufficient information and often have an erroneous notion that one strain could relieve all disease (van den Nieuwboer et al., 2016b). The large variety of probiotic products (available as food, dietary supplement or drug) together with the inability of companies to list the intended health indication on the product's packaging (as no health claims are approved for probiotics in Europe), create additional confusion for physicians and consumers. To this end, guidance documents that summarize and categorize available probiotic products per indication, as provided by Agamennone and colleagues (2018) for instance, can be of great assistance to foster adoption.

10.5 RESEARCH PRIORITIES FOR HEALTH CLAIM APPROVAL IN THE ADULT POPULATION

An increasing amount of clinical trials are being performed with probiotics, yet no health claim has been approved in Europe to date. Unable to communicate the intended health effects to consumers, this forms a prominent barrier to innovation which can (in part) be attributed to the wide range of potential therapeutic applications and a diluted distribution of research efforts. Here, we reviewed the current

clinical evidence of the two best documented probiotic strains (LGG & BB-12) to prioritize future research for health claim approval.

10.5.1 Key findings

A total of 92 clinical trials have been performed with LGG and BB12 in the adult population at the time of writing. Of these, 42 studies were performed in healthy adults or patient populations that are considered representative for effects in the general population. Bowel habit improvement (14 trials, 2240 subjects), immune support (24 trials, 375 subjects) and AAD prevention (7 trials, 300 subjects) were the most frequently studied indications, but 13 different health domains were identified. Chapter 9 shows that supplementation with LGG and BB-12 may promote human health and support the daily wellness of consumers in high priority areas. For instance, there is evidence that BB-12 beneficially affects stool frequency in populations with reduced stool frequency (without increasing diarrhoea). Furthermore, LGG appears to prevent AAD in patients treated for *H. pylori* infection. It is also suggested that both LGG and BB-12 (separately and in combination) support immune defence against pathogens in the upper respiratory tract.

While these results indicate that probiotic supplementation may support the daily wellness of consumers, the evidence is considered insufficient to support clear efficacy verdicts and substantiate health claim approval in Europe (EFSA Panel on Dietetic Products, 2011, 2013). This could be attributed to general difficulties in probiotic food research (Sanders et al., 2016), such as large interpersonal microbiota variability and the subtle effects of probiotics. However, it becomes increasingly evident that an overall lack of power in probiotic research trials is strong a diminishing factor for health claim substantiation. On average, the studies reviewed here included 52 participants per trial (with large variations), supporting the theory that 'pilotitis' (performing many small-scaled pilot studies that rarely enter sequential phase 3 trials) is a persistent barrier to probiotic innovation. Nonetheless, some of these health benefits have been acknowledged by other regulatory authorities, for instance, in Japan and Canada (He & Benno, 2011; Health Canada, 2015). It appears that the European criteria for the scientific substantiation of a health claim are particularly stringent (Binnendijk & Rijkers, 2013), and although this has expedited improved probiotic research quality over time, most (earlier) trials do not yet meet these standards.

10.5.2 Recommendations

To substantiate health claims in Europe, clinical trials need to evaluate the relationship between a specific probiotic and maintenance of good health or reduced risks of a disease in a healthy population. The claim should be substantiated with demonstratable changes in generally accepted biomarkers reflecting the risk of disease (EFSA, 2016). To stimulate probiotic research in this regard, Gibson et al (2011) have provided recommendations to design clinical trials and state that these should: (1) always formulate a precise and concrete hypothesis, and appropriate goals and parameters before starting a trial; (2) ensure they have sufficient sample size, such that they are adequately powered to reach statistically significant conclusions (taking into account adjustment for multiple testing), (3) ensure they are of appropriate duration and (4) focus on a single, primary objective and only evaluate multiple parameters when they are hypothesis-driven. These recommendations are valuable, but the scientific quality of a clinical trial is dependent on many more factors (i.e. appropriate monitoring, version control, audit-trials, quality assurance, adverse events reporting, and a-priori defining of hypotheses). Thorough recommendations are stipulated in ICH's Good Clinical Practice guidelines, which is considered the golden quality standard for pharmaceutical research trials (ICH Topic E6, R1). We urge that probiotic innovators follow these recommendations when designing quality controlled clinical trials to advance probiotic innovation throughout the entire valorisation cycle. Moreover, as the most prominent results for LGG and BB-12 were observed for AAD prevention (in patients treated for *H. pylori* infection), stool frequency improvement (in populations with reduced stool frequency) and immune defence in the upper respiratory tract, these health domains could be prioritized to fast track the health claim approval process.

10.6 VALIDITY & LIMITATIONS

To attain the objectives of this thesis, we utilized a mix method and interpretive approach using a combination of literature studies, quantitative surveys, systematic reviews, meta-analyses and in-depth interviews. Research methods were carefully selected, and methodologies were reviewed for each study to ensure their validity but may nonetheless present certain limitations that are discussed here and within the individual chapters of this thesis.

A meta-analysis was conducted in Chapter 5 to assess the efficacy of probiotic intervention for the prevention of constipation in elderly nursing home residents and to estimate the probiotic treatment effect. Results were obtained in a systematic manner for each study and parameters were carefully chosen based on the Rome IV criteria for functional constipation. However, as different strains, carrier matrices, and intervention periods were combined in this analysis, generalization of results should be done cautiously as the health effects of probiotics can differ significantly between species and strains and within different carrier matrices. In practice, this means that when selecting a probiotic for constipation prevention, one should carefully consider the individual studies within the meta-analysis to make an adequate informed choice on the preferred probiotic. For the purpose of this study, however, our results clearly provide an indication of probiotic efficacy and its potential to reduce health care expenditures in nursing homes.

In-depth, semi-structed interviews were performed in Chapter 6 to evaluate the impact of probiotic intervention on the quality of life of ulcerative colitis patients, thereby taking an interpretative, constructionist approach. This study method does not delineate experiences and perceptions by pre-defined or measurable categories and thus allows the inclusion of any relevant theme and is highly suited for the purpose of this study; gaining a deeper understanding of the perceptions and experiences of UC patients. In terms of clinical validity, however, this also means that statements regarding the impact of the intervention on quality of life rely on subjective experiences and interpretations. For future studies, we recommend combining these interviews with standardized and validated questionnaires such as the SF-36 and IBD Quality of Life Index (Guyatt et al., 1989).

To evaluate and quantify the perceptions of Medical Doctors and General Practitioners towards probiotics, quantitative surveys were utilized in Chapter 7 & 8. To ensure their internal validity, survey questions were first pilot tested with five medical doctors, whose feedback was incorporated into the questionnaire before being sent to participants. However, in the study with European physicians (Chapter 8), telephonic interviews were conducted rather than digital surveys (Chapter 7), aiming to improve the response rate while complying with the (then implemented) GDPR legislation. Telephonic interviews may introduce some bias, as the respondents can be more inclined to provide 'desirable' answers, or their opinions may be biased by the tone of the surveyor. Moreover, multiple choice questions in such surveys are provided in a certain order, where the first options could be selected more frequently as the interviewee is at that point unaware of the entire scope

of options. Survey administrators were therefore instructed to recall all options first, and then repeat them for choice selection in order to improve the validity of survey outcomes.

For the systematic review on the health benefits of LGG and BB-12 (Chapter 9), we evaluated their effects in a strain- and indication specific manner by systematically reviewing both the results and quality of the clinical trials at hand. While results clearly indicate that the intervention may have beneficial effects for certain indications, they are not quantified in terms of their combined treatment effect and significance. For future reference, clinical trials with LGG and BB-12 that report effects on stool frequency (in populations with reduced stool frequency) or AAD incidence (in populations treated for *H. pylori* infection) provide valuable grounds for meta-analyses, bearing in mind the limitations associated with the different carrier matrices and intervention periods that are used.

10.7 SUMMARY & CONCLUSION

Fermented foods have played a vital role in the advancement of human health for millennia. The microorganism residing within them are able to maintain or restore a balanced and diverse microbiota, inhibit the growth of pathogens, support immune defence and stimulate metabolism and nutritional intake (Chapter 1). In contemporary culture, we are able to isolate, culture and characterize these beneficial microbes to create specialized medicine or dietary supplements termed probiotics. Probiotic applications have an enormous potential to promote human health (Chapter 4, 5, and 9), as they are involved in numerous systemic, metabolic, neurological and immunological pathways. The health indications for which probiotics can be prescribed are therefore diverse, ranging from gut health to neurological disease, allergies and oncology (Chapter 9). Moreover, orally consumed probiotics have an excellent safety profile with few reported adverse events that make them a suitable intervention for adults, young children and elderly. Despite their potential, however, it appears that the innovation process for probiotics is hampered considerably, as relatively few probiotic strains are available commercially, their health claims are continuously rejected in Europe and there remains a lack of fundamental knowledge on probiotics and their interaction with the host (van den Nieuwboer et al., 2016b). We show in this thesis, that probiotic innovators are faced with several persistent challenges throughout the entire development cycle.

First and foremost, there is a clear lack of scientific substantiation of probiotic health effects and their underlying mechanisms of action, despite an increasing amount of (clinical) studies that are being performed. This is epitomized by the fact that no probiotic health claim has been approved by the EFSA to date. Even for the most substantiated probiotic strains, the combined evidence for a plethora of health indications is limited, and their mechanism of action is often poorly understood (Chapter 9). Moreover, both physicians and patients expressly state that they are awaiting more compelling evidence from research studies (Chapter 6, 7 and 8). Evaluating the health effects of probiotics in human studies is difficult in principle, because probiotic effects tend to be subtle, strain-specific and can vary substantially between individuals. Nonetheless, the quality of probiotic research studies is also generally suboptimal, especially compared with the 'pharma-standard' of controlled clinical research, exemplified by the frequent underpowered nature of these studies and lack of randomization (Chapter 4, 5 and 9). On the one hand, this is understandable as monetary investments in probiotic food studies are substantially less than investments in pharmaceutical research trials. This can be explained by the fact that unsubstantiated probiotics can also be freely sold on the open market, provided they are safe and produced according to appropriate quality standards, thereby creating a perceivably unfair competition that reduces the incentive to invest in costly clinical trials. Regardless, conducting multiple, successive, and high-powered efficacy studies (in healthy populations) in line with quality guidelines (such as ICH's GCP) and evaluating changes in generally accepted biomarkers reflecting the risk of a disease, are needed to substantiate health effects to European regulators that will facilitate their health claim approval. Obtaining this approval would generate a concise competitive advantage and will ultimately reduce the deleterious influence of unsubstantiated or 'pirate' probiotics. Moreover, scientific evidence and accompanying health claims may improve the perceptions of both physicians and patients towards probiotics, thereby improving public opinion, potentially increasing adoption and stimulating the promotion of human health.

While further efficacy evaluations in clinical trials are evidently needed to stimulate innovation, we also show in this thesis that the safety & accessibility of probiotics (Chapter 4 and 5), together with the influence of carrier matrices and production environments (Chapter 3), are underexposed within the probiotic industry. Indeed, probiotics have an excellent safety profile, but their adverse events are systematically reported in an imprecise, inconsistent and arguably incomplete manner (Chapter 4). Often a mere overall and unspecific safety statement is provided, but

the incidence of events is not reported. Another frequently underexposed aspect of probiotic innovation is the accessibility of the intervention for consumers and patients. Probiotics are widely available, in both supermarkets and pharmacies, but specialized probiotic formulations can be expensive and, as a dietary supplement, are generally not reimbursed by health insurers. It is therefore crucial to further evaluate the health economic potential of probiotics, as we established in chapter 5 for elderly nursing home residents. On a macroeconomic level, such data will help to convince insurance companies to reimburse-, and health institutions to adopt these interventions if they are able reduce other health care-related expenditures (Chapter 5). Moreover, probiotics are live microorganisms that are affected by their surroundings and need to be consistently monitored on their viability and functionality. We show that carrier matrices and production processes are able to alter probiotic functionality, gene expression or cell proliferation and therefore potentially affect their safety profile (Chapter 3), but critical factors contributing to these changes remain underexposed. Considering that some probiotics do not match the reported strains on the product's packing, or that some strains show altered gene expression when compared with the native strain, stresses the need for further (empirical and clinical) evaluation and improved quality control measures post-production and storage.

Lastly, we show that the legal access and utilization of probiotic microorganisms can be rather challenging (Chapter 2). Many successful production systems and technologies, including lactic acid bacteria in yoghurt and cheese production, have been transferred to other regions and nations over the years. A large share of genetic diversity used in conventional products is therefore of exotic origin. As people also frequently travel around the world and microbes move from the human body into the environment and other hosts, it raises the question of ownership and nationality of a microbe. It can therefore be difficult or even impossible to determine where a probiotic resource originated from, and consequently, with which country prior informed consent and mutually agreed terms need to be negotiated, making the legislative process for access and utilization of probiotics unattainable at times. There has also been much debate on whether genetic resources originating from the human microbiome (such as probiotics) should fall within the scope of such legislations. Many consider these to be human genetic resources that should not be covered by Access and Benefit Sharing legislations as it would be unethical for any government to have sovereign rights over such an important element of human physiology. Similarly, it is debated whether Genetic Sequence Data, vital for quick screening and discovery of new probiotics and their suitability for incorporation

into foods and medicine, should be covered by national or regional legislations. There are substantial opportunities to foster innovation within this domain by increasing uniformity and standardization, which would require increasing cooperation between industry, academia, regulators and providers of genetic resources, which can be stimulated in a multilateral system of facilitated access, similar to the Food and Agriculture Organization's (FAO) International Treaty on Plant Genetic Resources for Food and Agriculture.

Overall, we can see that the probiotic industry has shown tremendous growth over the past decades and is likely to retain this growth pattern. Intervention and supplementation with probiotics will therefore play an increasingly vital role in the promotion and maintenance of human health. We urge probiotic innovators to critically evaluate the quality and scope of their (proposed) clinical trials, increase cooperation with academia and regulators, and to continuously monitor and evaluate the quality of their strains, products and processes, in order to abate persistent challenges that hamper the probiotic innovation process.

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10