Accepted Manuscript

Biotechnology of health-promoting bacteria

François P. Douillard, Willem M. de Vos

PII: S0734-9750(19)30044-8
DOI: https://doi.org/10.1016/j.biotechadv.2019.03.008
Reference: JBA 7369

To appear in: *Biotechnology Advances*

Received date: 28 November 2018
Revised date: 15 February 2019
Accepted date: 11 March 2019

Please cite this article as: F.P. Douillard and W.M. de Vos, Biotechnology of health-promoting bacteria, Biotechnology Advances, https://doi.org/10.1016/j.biotechadv.2019.03.008

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Biotechnology of Health-Promoting Bacteria

François P. Douillard[1] & Willem M. de Vos[2,3,*] willem.devos@wur.nl

[1]Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

[2]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[3]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands

[*]Corresponding author.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abstract

Over the last decade, there has been an increasing scientific and public interest in bacteria that may positively contribute to human gut health and well-being. This interest is reflected by the ever-increasing number of developed functional food products containing health-promoting bacteria and reaching the market place as well as by the growing revenue and profits of notably bacterial supplements worldwide. Traditionally, the origin of probiotic-marketed bacteria was limited to a rather small number of bacterial species that mostly belong to lactic acid bacteria and bifidobacteria. Intensifying research efforts on the human gut microbiome offered novel insights into the role of human gut microbiota in health and disease, while also providing a deep and increasingly comprehensive understanding of the bacterial communities present in this complex ecosystem and their interactions with the gut-liver-brain axis. This resulted in rational and systematic approaches to select novel health-promoting bacteria or to engineer existing bacteria with enhanced probiotic properties. In parallel, the field of gut microbiomics developed into a fertile framework for the identification, isolation and characterization of a phylogenetically diverse array of health-promoting bacterial species, also called next-generation therapeutic bacteria. The present review will address these developments with specific attention for the selection and improvement of a selected number of health-promoting bacterial species and strains that are extensively studied or hold promise for future food or pharma product development.

Keywords: Applied Genomics, Gut Microbiota, Next-Generation Therapeutic Bacteria, Probiotics

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abbreviations

CRISPR	clustered regularly interspaced short palindromic repeats
GI	gastro-intestinal tract
GMO	genetically modified organism
LAB	lactic acid bacteria

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1. Introduction

The complexity of the human microbiota as well as its important implications for human health have been subject to a rapidly growing interest. A plethora of studies showed associations between the gut microbiota composition and infections, diseases or clinical conditions, paving the way to novel diagnostic approaches based on specific bacterial signatures found in patients (Flemer et al., 2018; Gilbert et al., 2016; Kahrstrom et al., 2016). Bacteria persisting in the human gut were found to interact with the host cells and other inhabitants of the gut as well as to play an immunomodulatory role (Hemarajata and Versalovic, 2013; Thaiss et al., 2016) and have systemic effects, among others *via* the gut-liver-brain axis (Collins et al., 2012; Sherwin et al., 2016). Moreover, it has been found that diet is one of the most important drivers of the microbiota composition and activity that in turn have an important systemic impact (Salonen and de Vos, 2014; Sonnenburg and Backhed, 2016). Of relevance for the food industry, the gut microbiota composition was shown to determine the impact of dietary interventions (Cotillard et al., 2013; Salonen and de Vos, 2014). Similarly, various studies have highlighted the contribution of the gut microbiota on the way individuals respond to specific drug therapies, bringing the interest in the intestinal microbiome in the realm of the pharma industry (Routy et al., 2018).

The biotechnology of functional food products has mainly focused on probiotics, defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). Typical traditional probiotics include lactic acid bacteria (LAB) and bifidobacteria (Figure 1) that are known to have a long history of safe use and are therefore well established on the probiotic market. The strong growth of the probiotic market has justified the need for further product development and diversification but also for the design and elaboration of the next-generation of live therapeutics and functional food products. Three main developments can be distinguished. The first includes intensified efforts to identify, characterize and select new probiotic candidates belonging to LAB and bifidobacteria with regard to their respective health-promoting properties to humans. In addition to the human body, *i.e.* gastro-intestinal tract (Goldin et al., 1992) or oral cavity (Beighton et al., 2008), a wider array of isolation

ACCEPTED MANUSCRIPT

sources have been explored and screened for probiotic candidates (Sornplang and Piyadeatsoontorn, 2016). Recent work on human gut health also resulted to a more rational and systematic approach based on genomics and other omics approaches, *in vitro* and *in vivo* assays to investigate the potential functional properties of these health-promoting bacteria (Figure 2). The second development relates to the use of genetic engineering tools since they offer novel strategies to enhance properties of health-promoting bacteria (Mays and Nair, 2018). While regulatory and safety aspects will not be addressed here, it is important to distinguish approaches that do not generate genetically modified organisms (GMOs), which are regulated strictly in Europe, versus the non-GMO approaches that lead to industrial strains that can be used without further specific limitations (Directive 2001/18/EC). The latter include bacterial strains improved by classical mutations or natural gene transfer systems (Bron et al., 2018). However, a different position is associated with the revolutionary CRISPR-Cas based genome-editing technology (Mougiakos et al., 2016). Presently, the regulations for the CRISPR-Cas technology are not uniformly adopted in all parts of the world and time will tell what the impact will be on bacterial engineering (Callaway, 2018). The final development involves the identification of novel gut bacteria not belonging to the traditional lactobacilli-bifidobacteria groups that emerged from the detailed characterization of the gut microbial composition (El Hage et al., 2017; O'Toole et al., 2017). These so-called next-generation therapeutic bacteria, including among others *Faecalibacterium prausnitzii* or *Akkermansia muciniphila,* display traits that are distinct from the ones reported in traditional probiotics (Cani and de Vos, 2017; Martin et al., 2018). Moreover, as these bacteria are natural gut commensals, it has been suggested that their use may result in longer colonization than traditional probiotic strains that do not colonize the human gut, where their effects persist only during a short period of time (Schmidt, 2013). Whether this is true remains to be seen since recent deep metagenomic sequencing studies of fecal microbiota transplants showed that there is considerable competition between strains of the same commensal species in the human gut (Li et al 2016).

The present review will discuss the increasing importance of the market of health-promoting bacteria and address the recent research developments of a selected number of bacterial species and strains that are widely commercialized worldwide and extensively researched. Finally, we will review novel health-

ACCEPTED MANUSCRIPT

promoting bacterial species that hold great potential for future developments of health-promoting functional products or therapeutics.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

2. Market development and production of health-promoting bacteria

The benefits of probiotic taxa have been well documented and their characterization at the molecular level is progressing (Sanders et al., 2018). However, only a limited number of approved claims have been associated with probiotic bacteria, possibly because of the way the health benefits are recorded and used in regulatory processes (Kleerebezem et al., 2018). In spite of these limitations, the market developments have shown a great interest by the consumer. Recent reports estimate the 2017 market for probiotic bacteria to be over 40 billion dollars and expect that to grow with an annual growth rate of approximately 7%, depending on the region in the world, to over 65 billion dollars in 2022 (Global Market Insight, 2018; Occams Business, 2017). This includes probiotics in foods and beverages with specifically the dairy segment to grow while some differences between growth of different species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* spp. were noted with growth rates of 8.5 %, 6% and 7.5 %, respectively (Global Market Insight, 2018). It should be noted that these and other market data are based on soft data that were not peer reviewed and hence the absolute numbers may vary but the trends are likely very real and have been recently reviewed (Taroncher-Oldenburg et al., 2018). In 2017, the total investment was over 500 million dollar, mainly focusing on therapeutics and around 10 % on diagnostics. This parallels the global market expectations for human microbiome-based therapeutics and diagnostics that reportedly is expected to grow to close to 1 billion dollar in 2024 (Markets and Markets, 2017; McKellar et al., 2017; Roots Analysis Business Research & Consulting, 2017). This includes next-generation therapeutic bacteria as sole culture or combination as well as their products.

Due to decades of experience, the cost-effective production of probiotics belonging to the canonical *Lactobacilli*, *Bifidobacteria and Streptococci* has been well established. However, as live bacteria are required in probiotic powder formulations, there has been considerable attention for preserving viability after freeze or spray-drying by enhancing stress resistance, optimizing protectants and improving the production process and dryer settings (Broeckx et al., 2016; Fu et al., 2018). Here, omics approaches such as proteomics are showing potential and together with flow-cytometry based techniques for assessing

ACCEPTED MANUSCRIPT

single cell viability offer options for improvements (Chiron et al., 2018; Mangiapane et al., 2015). Similarly, post-drying processes are being optimized and various encapsulation techniques have been described and reviewed (Coghetto et al., 2016; Sipailiene and Petraityte, 2018). While most of the canonical probiotic bacteria can tolerate and even use some oxygen, most of the next-generation therapeutic bacteria are strict anaerobes. Hence, the encapsulation and viable delivery of these may be pivotal (Marcial-Coba et al., 2018; van der Ark et al., 2017a).

The availability of genomes of probiotic bacteria allowed the development of genome-scale models that could support production optimization. However, this omics-based growth optimization for traditional probiotic bacteria has received only limited attention, in spite of the successful application of such models (Teusink et al., 2006). This may be explained by the empirical optimization methods that have led to present day biotechnological applications of the canonical probiotic bacteria. However, this does not hold for next-generation therapeutic bacteria that often have just been isolated from the human gut and here model-driven optimization has shown to be very productive (van der Ark et al., 2017b). For the strict anaerobe *Faecalibacterium prauznitzii*, this resulted in an improved understanding of its growth requirements for butyrate production and its metabolic cross talk with *Bifidobacterium adolescentis* (El-Semman et al., 2014). In the case of *Akkermansia muciniphila,* which was isolated as a mucus degrader, a genome-scale model led to the identification of growth parameters and the design of an animal-component free synthetic medium (van der Ark et al., 2018). This and other applications are expected to be instrumental in rapidly succeeding in producing these next-generation therapeutic bacteria to an industrial scale that is necessary for their application as health-promoting bacteria.

ACCEPTED MANUSCRIPT

3. Selection of traditional health-promoting bacterial species: a long and ongoing story

Historically and culturally, fermented foods have been associated with the human diet for a very long time, *i.e.* kimchi, kefir, yoghurt, sour milk or cheese (Shortt, 1999) and the development of food fermentation and preservation methods contributed to the domestication of some of these bacterial species (Douglas and Klaenhammer, 2010). Health-promoting properties of several of these microorganisms have been since well-documented but until recently, the detailed molecular mechanisms behind it and their roles on the gut microbiota were not well understood. Using classical and well-established screening methods, novel probiotic strains are now selected based on a number of well-defined criteria as established by FAO and WHO (Araya et al., 2002). These criteria relate to phenotypic/genotypic traits and functional properties, such as strain identification, stress tolerance, adherence to mucosa or intestinal epithelium, or safety (de Melo Pereira et al., 2018). Based on these recommendations, there is a growing number of novel strains or isolates from the canonical lactobacilli and bifidobacteria that have been classified as probiotics. In addition to generic criteria for selecting probiotics, functional properties of strains should be further studied for treating specific health conditions, *i.e.* cancer (Yamane et al., 2018), depression (Aizawa et al., 2016; Rudzki et al., 2018), obesity (Kim et al., 2018; Lee et al., 2018; Park et al., 2018) or diabetes (Khalili et al., 2018; Liu et al., 2018). Beside the probiotic properties of these strains, industrial considerations and production aspects are essential to allow the commercialization of these probiotics. Thus, traditional commercialized probiotics display phenotypic traits that are also compatible with industrials processes and that do not alter the organoleptic properties of the food products (Gueimonde and Sanchez, 2012).

Since the whole sequencing of the bacterial genome of *Haemophilus influenza* (Fleischmann et al., 1995), there has been literally a revolution in the field of genome sequencing technologies. The development of next-generation whole genomic sequencing allowed in-depth characterization of the coding capacity of probiotic strains, unveiling genes associated with probiotic traits. Functional genomics brought insights into molecular mechanisms and mode of action of probiotic strains, resulting in the identification novel sets of genes associated with probiotic functions. Notable probiotic and/or widely commercialized strains were

ACCEPTED MANUSCRIPT

among the first lactobacilli and bifidobacteria to be sequenced but were soon followed with the genome sequencing of a plethora of other isolates, as this is illustrated by the number of deposited genomes of traditional probiotics (Table 1). This provided insights into the phylogenetic diversity of LAB and bifidobacteria. For example, *Lactobacillus rhamnosus, Lactobacillus salivarius* or *Lactobacillus sakei* were found to have a high intra-species diversity (Ceapa et al., 2016; Chaillou et al., 2013; Douillard et al., 2013; Harris et al., 2017), underlying that functional properties towards the host are very much strain-dependent. Moreover, following the deep analysis of hundreds of *Lactobacillus* genomes, their taxonomy is being revised and this may lead to a new nomenclature for the presently 12 phylogroups that have been detected (Salvetti et al., 2018). In contrast, the diversity of species like *Bifidobacterium animalis* subsp. *lactis* is singularly low, indicating that the strains of this species share a recent ancestor (Milani et al., 2013) and therefore may display comparable phenotypic traits. Initial comparative genomic analysis at the species levels also provided important data on how to select new isolates in a given species by identifying sets of genes that are known to be associated with probiotic functions, such as adherence or stress tolerance. For example, in *Lactobacillus rhamnosus*, comparative genomic analysis of two strains (GG and Lc705) led to the identification of a gene cluster (*spaCBA-srtC1*) coding for sortase-dependent pili that adheres to the intestinal mucosa, which was then further confirmed by *in vitro* assays and animal models (Kankainen et al., 2009). Similarly, in *Bifidobacterium breve* UCC2003, genes encoding type IVb tight adherence (Tad) fimbriae were uncovered (O'Connell Motherway et al., 2011). In *Lactobacillus salivarius* strain UCC118, genes coding for mucus-binding LPXTG proteins were identified and assessed by adhesion assays (van Pijkeren et al., 2006). The number of genes associated with probiotic functions is constantly growing and brings novel insights into the mode of action of health-promoting bacteria in the gut (Lebeer et al., 2018; Lebeer et al., 2008). Their availability constitutes important information for the selection of probiotic candidates. It includes dozens of genes associated with survival in the GI tract, host-bacteria interaction, immunomodulation, antimicrobial activity or pathogen control, for a recent review see (Lebeer et al., 2018). In addition to selecting for probiotic traits, genome sequencing allows the identification of genes associated with virulence and antibiotic resistance (safety assessment) and an accurate taxonomic

ACCEPTED MANUSCRIPT

identification of the strains (Figure 3), since in some cases, 16S rRNA gene sequencing is not suitable for species determination (Torriani et al., 2001). By screening and selecting *in silico* strains, larger pool of isolates can be analyzed prior to any wet lab analyses, preventing extensive and fastidious laboratory screening. Genomic sequencing of probiotic candidates do, however, have technical bottlenecks, since there is not always an evident association between gene and function, justifying need to conduct phenotypic characterization and complementary omics approaches, like transcriptomics, metabolomics, methylomics or proteomics to further comprehend gene activity under various culturing conditions and more broadly mode of actions of health-promoting bacteria, *i.e.* heat shock (Rezzonico et al., 2007), bile salt resistance (Ruiz et al., 2012), acid stress (Koponen et al., 2012) or anti-microbial compound production (Riboulet-Bisson et al., 2012).

4. Tools and techniques for improvement of health-promoting bacteria

Criteria for selecting probiotic bacteria isolated from various ecological niches are well established and in conjugation with omic approaches, this allows high-throughput and efficient mining of novel isolates with probiotic potential. However, existing probiotic strains were shown to have limited effects in some cases, as previously reported (Bomba et al., 2002; Karimi and Pena, 2008; Koo et al., 2012). The increasing knowledge on the mode of action of probiotic bacteria in the gut towards the host and its associated microbiota now offers a basis for the rationale design of bio-engineered probiotic strains with tailored functional properties. The improvement, alteration or acquisition of phenotypic traits in existing probiotic strains can positively impact their performance and function in the gut. Thus, bio-engineered traits may relate to colonization in the gut, *i.e.* mucosal adherence or acid stress resistance, immuno-modulation, antimicrobial activity or production/display of enzymes or structural proteins, metabolites or active compounds in the gut (Figure 4). Bio-engineering of probiotic strains also offers the possibility to delete problematic genes, *i.e.* antibiotic resistance and virulence genes that were initially reported in isolates with probiotic potential. The need for this may increase as various *Lactobacillus* spp. have been found to carry

ACCEPTED MANUSCRIPT

antibiotic-resistance genes that are potentially transferrable (Campedelli et al., 2018). Moreover, many intestinal anaerobes are also antibiotic resistant (see below), possibly due to exposure of the host to the widespread use of antibiotics. Hence, there is a need to efficiently inactivate these in all health-promoting bacteria that reach the consumer.

Optimization of existing probiotic bacteria can occur in various ways. A possible overlooked approach is the use of fermentation optimization. A recent example of this is the so-called upgrading of the fermentation of *Propionibacterium freudenreichii*, a less-often used probiotic strain and a natural producer of vitamin B12 (Kajander et al., 2008; Piwowarek et al., 2018). This was realized in such a way that the cells of *P. freudenreichii* strain W200 now have adequate quantities of this vitamin as to retain an EFSA validated health claim (Winclove Probiotics, NL). Other well-known approaches include selection by random mutagenesis, forced evolution, CRISPR-Cas-mediated genome editing or use of cryptic plasmid and food-grade vectors (Derkx et al., 2014). These and others allow tailoring of the coding capacity of probiotic strains with respect to their intended applications. However, it has to be kept in mind that some technologies generate bacterial variants that may be considered in some countries as genetically modified organisms (GMOs). Since the application of such GMOs is highly restricted, non-GMO approaches have a wider range of applications in industry. In *Lactobacillus rhamnosus* strain GG, random mutagenesis was carried out to generate non-GMO variants devoid of mucus-binding pili as a result of either mutations within the pilus gene cluster or large chromosomal rearrangements (Rasinkangas et al., 2014). Following a similar approach, *L. rhamnosus* GG derivatives with a higher mucus adherence were also obtained (Rasinkangas, 2016). This exemplifies how the performance of a specific trait can be altered to generate a range of phenotypes for the same strain. Adhesion ability and tolerance to glucose-induced carbon catabolite repression in *L. plantarum* strains were improved using random mutagenesis (Seme et al., 2017; Zhao et al., 2017). Random mutagenesis is a rather unspecific approach, since secondary/unwanted mutations also occur with possible and deleterious impact on the phenotype. However, in combination with high throughput next-generation sequencing the undesired strains can be easily detected and removed from the selection process (Rasinkangas et al., 2014). A more direct approach is the use of GMO

ACCEPTED MANUSCRIPT

approaches that have also been extensively used to improve the adaptation of strains to the gut environment. Thus, the tolerance to acid and high osmolarity in *L. salivarius* strain UCC118 was improved by expressing *betL* coding for betaine uptake system in *Listeria monocytogenes* (Sheehan et al., 2006). On the other hand, GMO approaches have also used for the display of antigens or antibodies as recently reviewed (Michon et al., 2016; Szatraj et al., 2017) and the production of recombinant proteins, *i.e.* human recombinant phenylalanine hydroxylase or interleukin IL-10 in *L. plantarum* (Cai et al., 2016; Ramirez et al., 2017). The use of food-grade expression systems based on DNA homologous to the hosts or other food bacteria is typically preferred, since antibiotic resistance markers need to be avoided. Such an approach has been successful, as evidenced in *L. lactis* expressing the gene for elafin (a protease inhibitor) for the treatment of inflammatory bowel diseases (Motta et al., 2012) or secreting interleukin IL-10 for food allergy management (Robert and Steidler, 2014). An extensive range of LAB and bifidobacteria have been so far successfully bio-engineered, including among others, species like *L. paracasei*, *L. lactis*, *L. salivarius* or *L. reuteri* (Mathipa and Thantsha, 2017).

Until now, GMO techniques have been mostly developed to modify the properties of probiotic bacteria as to better comprehend their mode of action in the gut. Only in few studies, bacterial strains were purposely engineered using conventional strategies for conducting human interventions (Robert and Steidler, 2014). Over the last few years, the CRISPR-Cas genome editing tool has revolutionized research in life sciences and is about to have a similar impact on the bio-engineering of probiotic strains. The CRISPR-Cas system was successfully implemented in lactobacilli and bifidobacteria, although it may be differently effective among strains of the same bacterial species (Leenay et al., 2018). The potential applications of the CRISPR-Cas mediated engineering system in probiotic bacteria highlight the promise for significant advances in the development of health-promoting bacteria with enhanced probiotic properties or specifically tailored for a given application (van Pijkeren and Barrangou, 2017). The regulatory status of the revolutionary CRISPR-Cas based genome-editing technology is, however, presently controversial and being subject to different regulatory regimes in various parts of the world (Mougiakos et al., 2016).

ACCEPTED MANUSCRIPT

5. Microbiome-based next-generation therapeutic bacteria

Most of the insight into the human microbiome has been based on 16S rRNA or metagenome analysis, only providing information on the gut microbiota at the genus or group level. However, when the bacterial signatures are very marked, associations with health parameters can be made and may identify potential next-generation species. Only when cultured representatives are available, causal relations can be established. Presently, type strains of over 1000 species have been described and deposited in accessible strain collections (Rajilić-Stojanović and de Vos, 2014). This illustrates the fact that there is some choice and in many cases, various strains from the same bacterial species are available allowing approaches as discussed for the screening of novel probiotic microbes (Figure 3). Following the selection of an appropriate strain, safety and causality studies are often performed in mouse models even while it is known that the mouse microbiota differs considerably from that in human (Hugenholtz and de Vos, 2018). However, these models have the additional advantage to provide insight in the safety of the candidate strains. This has been the case with several of the health-promoting anaerobes belonging to the major phyla in the human gut, including the *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia* (Figure 1). While the mechanisms of action of these next-generation therapeutic bacteria are being studied in detail, several produce butyrate and the production of this short chain fatty acid is a characteristic that is not found among the canonical probiotic strains and may explain some of the health benefits (Schroeder and Backhed, 2016). Examples are discussed below with specific attention for their characteristics, safety aspects, and application potential. In addition, in some cases comparisons, are made with the approaches discussed for new probiotic strains.

Bacteroidetes are true Gram-negative bacteria that have high abundance in the human intestinal tract of approximately 20-40 % (Arumugam et al., 2011). This phylum comprises various genera, the most notable being *Bacteroides* for which there are at least 25 species validly described, including some potentially pathogenic ones (Rajilić-Stojanović and de Vos, 2014). Some *Bacteroides* spp. have found to be, contain structures that are immunochemically identical to the alpha-Gal epitope or the Thomsen-Friedenreich (TFα) antigen, a tumor-specific carbohydrate antigen (Henderson et al., 2011). Interest in a potential new

ACCEPTED MANUSCRIPT

therapeutic that could evoke an immune reaction focused on *B. xylanisolvens*, a strict anaerobe which degrades xylan and other sugars into acetate, propionate and succinate (Chassard et al., 2008). Likely based on the notion that non-viable *B. xylanisolvens* may still contain the desired structure and induce the desired immune response, an elaborate safety assessment was performed for pasteurized cells and it was concluded that heat-treated milk products fermented with the type strain *B. xylanisolvens* DSM 23964 would be safe for use in humans under certain conditions (EFSA NDA Panel, 2015). A subsequent human trial confirmed that pasteurized *B. xylanisolvens* DSM 23964 is capable of inducing immunoglobulin M serum antibodies against the TFα antigen (Ulsemer et al., 2016). However, details on the application of *B. xylanisolvens* DSM 23964 in either the food or pharma space are missing. It is of interest to note though that the safety evaluation *B. xylanisolvens* DSM 23964 was performed in the knowledge that its genome contains a functional *cepA* gene coding for penicillin resistance – as this gene is widely spread in *Bacteroides* spp., apparently not located on a conjugative element, and the cells are pasteurized, it was concluded that this is not a risk factor (EFSA NDA Panel, 2015). Future studies will show what benefits the *B. xylanisolvens* pasteurized product will have, what mode of action could explain the results, and what markets it will address.

One of the largest group of intestinal bacteria in both abundance and species richness is that of the *Firmicutes* (Qin et al., 2010). This phylum also contains the *Lactobacillicaeae* that include canonical probiotic strains but their abundance in the intestinal tract is very modest with 1% at most. Much more abundant are a wide range of anaerobes that among others belong to the class of *Clostridia* and include *Clostridiaceae, Ruminococcaceae, Lachnospiraceae* and *Christensenellaceae.* The *Clostridia* make up a significant part and were named after the *Clostridium* type species *C. butyricum* that was discovered over 100 years ago (Rajilić-Stojanović and de Vos, 2014). *C. butyricum* is a spore-forming anaerobe capable of producing butyrate, butanol and 1,3-propanediol, while vigorously producing hydrogen. Interestingly, the non-toxigenic *C. butyricum* strain MIYAIRI 588 has been widely marketed in Asia and has it was shown to reduce symptoms in infants with antibiotic associated diarrhea (Seki et al., 2003). Moreover, this strain was also able to reduce growth of pathogenic *Clostridium difficile* in a rodent model (Oka et al., 2018).

ACCEPTED MANUSCRIPT

Interestingly, *C. butyricum* MIYAIRI 588 is not a gut commensal as it has been isolated from a Japanese soil sample. While its genome has not been reported yet, the strain carries a plasmid that has been characterized at the sequence level and was found to encode a butyricin-like bacteriocin with bactericidal properties (Nakanishi and Tanaka, 2010). This may at least partially explain the effectiveness of *C. butyricum* MIYAIRI 588 but so far no survival or colonization studies in human have been reported. The genome of a related strain of *C. butyricum,* strain DKU-01 isolated from infant feces, has been determined and found to encode the utilization of fructo-oligosaccharides but no functional studies of this strain have been performed yet. A recent review has addressed potential issues related to safety of *C. butyricum* strains as these are often found in infants with necrotizing enterocolitis (Cassir et al., 2016). Nevertheless, since *C. butyricum* MIYAIRI 588 has a long history of safe use, it is allowed on the EU market (Commission 2014/907/EU).

Bacteria belonging to the *Ruminococacceae* and *Lachnospiraceae*, previously known as Cluster IV and Cluster XIVa Clostridia, respectively, form the most abundant intestinal anaerobes and include some representatives that have been studied as next-generation health-promoting bacteria. *Faecalibacterium prausnitzii* belonging to the *Ruminococacceae* has received most attention since it is among the most prominent bacterial groups in the intestinal tract, varying from a few to fifteen percent of the total bacterial population (Lopez-Siles et al., 2017). Likely because of its abundance and relatively easy detection, there have been an impressive number of studies describing a reduced abundance of bacteria related to *F. prausnitzii* in patients with several human diseases, notably ulcerative colitis and Crohn's disease (Miquel et al., 2013). However, recent sequencing studies showed that there are at least three different *F. prausnitzii* phylogroups, likely representing different species (Benevides et al., 2017). This complexing factor illustrates the difficulties of the present state of the art of the intestinal microbiome research. Strain *F. prausnitzii* A2-165 was studied first in an set of elegant experiments that have shown causality of its cells as well as culture supernatant that were capable of protecting mice in a colitis model (Sokol et al., 2008). Of interest has been the observation that a proteinaceous fraction of *F. prausnitzii* A2-165 was capable of partly reproducing this effect opening the way for more detailed molecular and mode of action studies (Quevrain

ACCEPTED MANUSCRIPT

et al., 2016). In addition, the biofilm forming *F. prausnitzii* strain HTF-F has been used to show anti-inflammatory effect *in vitro* (Rossi et al., 2016). Recently, a comprehensive set of comparative studies were described for a dozen *F. prausnitzii* isolates showing considerable differences in oxygen sensitivity, antibiotic resistances and immunomodulatory properties (Martin et al., 2017). This is in line with another study that showed variability in improving barrier function of various *F. prausnitzii* strains and noted that strains A2-165 and HTF-F did not affect this parameter (Maier et al., 2017). All these findings are reminiscent of probiotic bacteria that in most cases show strain–specific effects and illustrate the heterogeneity of the *F. prausnitzii* group. However, the detailed comparative studies are of great interest since they offer the possibility to select strains that are effective, do not carry antibiotic resistance genes, and can be grown on a large scale as to allow their production for human intervention, very much along the same lines as novel probiotic strains (Figure 3). In this context it is of interest to note that the oxygen-sensitive *F. prausnitzii* strain A2-165 could be kept alive at ambient oxygen concentration when formulated with the antioxidants cysteine and riboflavin plus the cryoprotectant inulin (Khan et al., 2014). The safety of *F. prausnitzii* should be further addressed as it has been recently suggested (Brodmann et al., 2017).

Following a comprehensive analysis of the microbiota of a large twin cohort, it was found that bacteria belonging to the *Christensenellaceae* were highly heritable and linked to a lean phenotype (Goodrich et al., 2014). The first and so far only isolate of *Christensenellaceae* is *Christensensella minuta* DSM 22607, a small and non-spore forming anaerobe that is capable of producing butyrate from a few sugars. While it belongs to the *Firmicutes*, it stains like a Gram-negative bacterium, a feature also observed for *F. prausnitzii*. In a single experiment, this strain was grown and used to amend a fecal sample from an obese subject resulting in a reduced weight gain of the mice. This interesting finding sparkled interest in *C. minuta* although no repeat studies of the successful spiking have been yet described. Its 2.5 Mb- genome has been analyzed (Rosa et al., 2017; Yang et al., 2018) (Table 1). Detailed analysis revealed it to contain several resistance genes, including the *tetW* gene encoding tetracycline resistance. Whether *C. minuta* is a real Gram-negative bacterium needs to be addressed but it has been found produce an unusual lipopolysaccharide (LPS) that has limited agonist activity in *ex vivo* models as compared to the LPS of the well-known Gram-negative

ACCEPTED MANUSCRIPT

Escherichia coli (Yang et al., 2018). A recent report described *C. minuta* to be present together with *Desulfovibrio* isolate in the blood of an appendicitis patient (Alonso et al., 2017). The presence of LPS, and the presence of potentially transferable resistance genes may indicate that a detailed toxicity analysis is needed before human trials of this interesting species could be considered.

Causality has been addressed in the development of *Eubacterium hallii* as a next-generation therapeutic microbe by using fecal microbiota transplantation. Metabolic syndrome adults improved their insulin sensitivity significantly only after a duodenal infusion with fecal microbiota from a healthy donor but not with their own microbiota (Vrieze et al., 2012). Detailed analysis showed that the microbiota in the upper intestinal tract microbiota showed more differences than that in the colon. It was observed in duodenal biopsies that bacteria related to *Eubacterium hallii* were relatively increased in subjects receiving a healthy donor transplantation as compared to autologous controls (Vrieze et al., 2012). *Eubacterium* is a genus that is clearly not monophyletic and while some of its species belong in the *Ruminococcaceae*, the butyrate-producing *E. hallii* has a different phylogenetic position and is grouped in the *Lachnospiraceae* (Rajilić-Stojanović and de Vos, 2014). *E. hallii* is a metabolically highly versatile bacterium that is not only capable of producing butyrate from glucose and other sugars but also converts both D- and L- lactate to butyrate in presence of acetate (Duncan et al., 2004). Moreover, recently *E. hallii* has also been reported to produce propionate from 1,2-propanediol, an intermediate that can be generated from rhamnose and fucose (Engels et al., 2016). Following the human fecal microbiota transplant study, a trial with obese and diabetic mice was performed with *E. hallii* strain L2-7 that had been isolated from a healthy infant (Duncan et al., 2004). This resulted in improvement of insulin sensitivity and adiposity in a dose-dependent way while active cells of *E. hallii* L2-7 were found to increase fecal butyrate concentrations, modify bile acid metabolism and reduced liver triglyceride levels (Udayappan et al., 2016). Moreover, expression studies showed that notably small intestinal rather than colonic genes were affected by live. This can be rationalized since the upper intestinal tract is much less densely populated than the colon and contains microbes that mainly produce lactate and acetate (Zoetendal et al., 2012). The conversion in the small intestinal of lactate and acetate into butyrate could be a mechanism by which *E. hallii* L2-7 could improve

ACCEPTED MANUSCRIPT

metabolic health, in line with its potential action in the fecal transplantation trial. New insight based on genomic and physiological characteristics has led to adapt the phylogenetic position of *E. hallii* and this has now been classified into the genus *Anaerobutyricum* with two species, *A. hallii* including the type strain and *A. soehngenii,* including strain L2-7 (Shetty et al., 2018). Butyrate production from lactate and acetate is only mediated by a small group of intestinal bacteria, including these *Anaerobutyricum* spp. as well as the related *Anaerostipes* spp. and this has recently been studied in detail revealing a new and characteristic pathway involved in this conversion (Shetty, 2019). These specialized butyrate producers may have all potential for the development as next-generation therapeutic bacteria, notably for the treatment of subjects with metabolic syndrome to prevent the development of type 2 diabetes.

While most industrial probiotic strains have been derived from human, some also have an animal origin. This is notably the case for *B. animalis* subsp. *lactis* BB-12 that is used worldwide in food products. A similar situation is the case for *Butyricicoccus pullicaecorum* that was described a decade ago as a new species when several strains were isolated from the cecum of oligo fructose-fed chicken and found to produce butyrate from variety of sugars (Eeckhaut et al., 2008). Initially described as belonging to the *Ruminococcaceae*, *B. pullicaecorum* also has been grouped in the Unclassified Clostridia, also belonging to the *Firmicutes* (Rajilić-Stojanović and de Vos, 2014). Following the observation that the average number of *Butyricicoccus* spp. in stools from patients with inflammatory bowel disease was significantly than that of healthy subjects, the *B. pullicaecorum* type strain 25-3^T was selected for further analysis**.** A remarkable protective effect was observed upon oral administration of this strain in a colitis mouse model (Eeckhaut et al., 2013). Favorable rat safety trials have been reported of *B. pullicaecorum* strain 25-3^T and its partial genome sequence was determined (Steppe et al., 2014). This showed the presence of a complete *tetW* gene but unexpectedly the tetracycline resistance of the type strain was low although other antibiotic resistances were reported (Steppe et al 2014). To further its potential application, a safety trial in healthy adults was recently reported that showed encapsulated *B. pullicaecorum* strain 25-3^T to be safe and well tolerated in human (Boesmans et al., 2018). While there was no impact on the overall microbial community composition, unexpectedly no accumulation of the treatment genus over the intervention study was

ACCEPTED MANUSCRIPT

observed. However, this may be due to the relatively high baseline level of the genus *Butyricicoccus*. As this is the case with many next-generation therapeutic bacteria, there are no media that can be used to selectively enumerate the administered strain. However, deep metagenomic sequencing in combination with advanced computational methods can allow strain level analysis as recently reported (Li et al 2016). Alternatively, the strain may have lysed during transit but this is unlikely since its encapsulation was tested thoroughly (Eeckhaut et al., 2014). It is of great interest to follow the further development of *B. pullicaecorum* strain 25-3^T as a candidate for next-generation therapeutic bacteria that could have clinical benefits in inflammatory bowel disease.

Recent years have seen a rapidly increasing attention in *Akkermansia muciniphila*, the sole representative of the *Verrucomicrobia* in the human intestinal tract that was discovered in a search for mucus-degrading commensals with the idea that these would interact beneficially with the host (Derrien et al., 2004). Some of this attention can be explained as *A. muciniphila* can be detected easily because of its deeply rooted phylogenetic position. Hence it stands out in many microbiota profiling studies and the presence of *A. muciniphila* has been associated with health in dozens of studies (Derrien et al., 2017). Germ-free mice mono-associated with *A. muciniphila* showed marked metabolic and immune signaling in the colon while in the same analysis *L. plantarum* was signaling mostly in the upper intestinal tract (Derrien et al., 2011). Subsequent studies demonstrated that living *A. muciniphila* cells were capable of protecting mice from diet-induced obesity and improved barrier function (Everard et al., 2013). Many studies confirmed this and the improvement of colonic barrier function by *A. muciniphila* has been reproduced in many different laboratories around the world (Cani and de Vos, 2017). This supported the original hypothesis of host interaction and is in line with the abundance of *A. muciniphila* in the colon where it can reach in healthy subjects levels of around 2-5 %, while phylogenetic and genomic analysis showed that present isolates all belong to the same species (Belzer and de Vos, 2012; Geerlings et al., 2018). The mode of action has been studied using molecular approaches and identified a specific outer membrane protein Amuc_1100 that was signals to the TLR2-receptor and is capable of reproducing the effect (Ottman et al., 2016; Plovier et al., 2017). The 30 kDa Amuc_1100 protein is relatively heat stable explaining why also pasteurized cells of *A.*

ACCEPTED MANUSCRIPT

muciniphila were capable of improving barrier function and protecting mice from diet induced obesity (Plovier et al., 2017). This opens avenues to produce stable and inactivated *A. muciniphila* cells that could be used as supplements or other formulations. *A. muciniphila* could be produced in animal-component free media (describe above) and thus produced cells were used in human trials to assess its safety making this unusual commensal one of the most promising candidate as a next-generation therapeutic (Plovier et al., 2017). In addition, the Amuc_1100 protein could serve well as a vehicle to induce barrier function in a clinical settings and this is currently under investigation.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

6. Concluding remarks

Current probiotic-marketed products mainly consists of bacterial strains belonging the canonical lactobacilli-bifidobacteria group. These strains have been isolated and further selected for their natural probiotic traits and their compatibility with industrial processes and product formulation. Revolutionary bio-engineering tools, such as the CRISPR-Cas system, and the explosion of microbiomics are now challenging this established scheme and will contribute hand-in-hand to the rational design of bacterial strains with enhanced/tailored probiotic properties compared to their wild-type counterparts and the emergence of a whole new array of microbiota-derived species or strains that harbor traits absent in traditional probiotics. These major developments in the field of health-promoting bacteria hold great promise for the future development of functional products with better performance and tailored functional properties that are targeting very specific applications. Moreover, the microbiome-based next-generation therapeutic bacteria are developing rapidly, target a great variety of new health functions, and hence hold great potential for the food and pharma industry.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table

Table 1. List of common bacterial species whose members are harboring health-promoting properties. Their respective phylogenetic relatedness is shown in Figure 1. For each species is indicated the number of deposited closed genomes and assemblies as retrieved from in NCBI databases on the date of 27th October 2018.

	Bacterial Species	Closed Genome	Genome Assemblies	Genome Example	Origin	References
Traditional probiotic bacteria						
	Lactobacillus acidophilus	6	36	NCFM	Human intestine	(Altermann et al., 2005)
	Lactobacillus brevis	13	53	ATCC 367	Undefined	(Makarova et al., 2006)
	Lactobacillus casei	6	24	BL23	Cheese	(Maze et al., 2010)
	Lactobacillus helveticus	13	50	DPC 4571	Cheese	(Callanan et al., 2008)
	Lactobacillus jensenii	1	18	SNUV360	Vagina	(Lee et al., 2017)
	Lactobacillus paracasei	21	109	N1115	Fermented milk	(Wang et al., 2014)
	Lactobacillus plantarum	67	304	WCFS1	Human saliva	(Siezen et al., 2012)
	Lactobacillus reuteri	12	118	DSM 20016	Human intestine	(Sun et al., 2015)
	Lactobacillus rhamnosus	16	151	GG	Human intestine	(Kankainen et al., 2009)
	Lactobacillus salivarius subsp. *salivarius*	8	83	UCC118	Human intestine	(Claesson et al., 2006)
	Lactococcus lactis subsp. *lactis*	37	150	IL1403	Cheese	(Bolotin et al., 2001)
	Bifidobacterium animalis subsp. *lactis*	20	50	BB-12	Infant feces	(Garrigues et al., 2010)
	Bifidobacterium bifidum	9	58	BGN4	Infant feces	(Yu et al., 2012)
	Bifidobacterium breve	38	93	UCC2003	Infant feces	(O'Connell Motherway et al., 2011)
	Bifidobacterium longum	20	172	JDM301	Infant feces	(Wei et al., 2010)
Next-generation therapeutic bacteria	*Propionibacterium freudenreichii*	23	45	CIRM-BIA129	Cheese	(Falentin et al., 2016)
	Akkermansia muciniphila	2	56	ATCC BAA-835	Human feces	(van Passel et al., 2011)
	Bacteroides xylanisolvens	0	18	XB1A	Human feces	Direct submission
	Butyricicoccus pullicaecorum	0	3	25-3(T)	Chicken cecal content	(Steppe et al., 2014)
	Christensenella minuta	0	3	DSM22607	Human feces	(Rosa et al., 2017)
	Clostridium butyricum	5	20	DKU-01	Infant feces	(Mo et al., 2015)
	Eubacterium hallii	1	9	L2-7	Infant feces	(Shetty et al., 2017)
	Faecalibacterium prausnitzii	4	40	A2-165	Human feces	(Benevides et al., 2017)

ACCEPTED MANUSCRIPT

Figures

Figure 1. Rooted phylogenetic tree of bacterial species that have been shown to display health-promoting properties. The tree was generated using the online analysis resource from PATRIC (Wattam et al., 2017) and rendered using Phylo.io (Robinson et al., 2016). The present tree aims at illustrating the growing phylogenetic diversification of bacterial species that are positively associated with human health rather than providing an overview of all bacterial taxa that are marketed as probiotics. For clarity, not all known and relevant bacterial species were included. Legend: blue, traditional probiotics; green, next-generation probiotics.

Figure 2. Biotechnology of health-promoting bacteria: an overview. Novel insights into the gut microbiota and the parallel development of new technologies are opening new avenues for the use and enhancement of existing and novel probiotic species/strains.

Figure 3. Workflow for the selection of health-promoting bacteria based on omic approaches and *in silico* predictive models.

Figure 4. Bio-engineering health-promoting bacteria to increase probiotic potential and impact on gut health. Through the use of various genetic engineering or mutation-selection strategies, the coding capacity of probiotics can be altered to further improve colonization, stress resistance, stability, quorum-sensing, host interaction or to produce active compounds and nutrients, *i.e.* short chain fatty acids and vitamins. Such approaches would also allow the development of tailored probiotics specific to a given application, disease or health condition.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Competing interests

WMdV is co-founder of A-Mansia Biotech SA Brussels developing *Akkermansia muciniphila*-based products and Caelus Health BV Amsterdam developing *Eubacterium hallii* and other butyrate-producing bacteria.

Acknowledgments

WMdV was supported by the Netherlands Organization for Scientific Research (SIAM Gravitation grant 024-002-002 and the unrestricted Spinoza Award) as well as the University of Helsinki. FPD was supported by the University of Helsinki. Due to space limitations and time constraints, we apologize for not citing all research work relevant to the topic covered by the present review.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

References

Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Ota, M., Koga, N., Hattori, K., Kunugi, H., 2016. Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. J Affect Disord 202, 254-257.

Alonso, B.L., Irigoyen von Sierakowski, A., Sáez Nieto, J.A., Rosel, A.B., 2017. First report of human infection by *Christensenella minuta*, a gram-negative, strickly anaerobic rod that inhabits the human intestine. Anaerobe 44, 124-125.

Altermann, E., Russell, W.M., Azcarate-Peril, M.A., Barrangou, R., Buck, B.L., McAuliffe, O., Souther, N., Dobson, A., Duong, T., Callanan, M., Lick, S., Hamrick, A., Cano, R., Klaenhammer, T.R., 2005. Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. Proc Natl Acad Sci U S A 102(11), 3906-3912.

Araya, M., Morelli, L., Reid, G., Sanders, M.E., Stanton, C., Pineiro, M., Ben Embarek, P., 2002. Guidelines for the evaluation of probiotics in food. Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food, 1-11.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Nielsen, H.B., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E.G., Wang, J., Guarner, F., Pedersen, O., de Vos, W.M., Brunak, S., Dore, J., Meta, H.I.T.C., Antolin, M., Artiguenave, F., Blottiere, H.M., Almeida, M., Brechot, C., Cara, C., Chervaux, C., Cultrone, A., Delorme, C., Denariaz, G., Dervyn, R., Foerstner, K.U., Friss, C., van de Guchte, M., Guedon, E., Haimet, F., Huber, W., van Hylckama-Vlieg, J., Jamet, A., Juste, C., Kaci, G., Knol, J., Lakhdari, O., Layec, S., Le Roux, K., Maguin, E., Merieux, A., Melo Minardi, R., M'Rini, C., Muller, J., Oozeer, R., Parkhill, J., Renault, P., Rescigno, M., Sanchez, N., Sunagawa, S., Torrejon, A., Turner, K., Vandemeulebrouck, G., Varela, E., Winogradsky, Y., Zeller, G., Weissenbach, J., Ehrlich, S.D., Bork, P., 2011. Enterotypes of the human gut microbiome. Nature 473(7346), 174-180.

Beighton, D., Gilbert, S.C., Clark, D., Mantzourani, M., Al-Haboubi, M., Ali, F., Ransome, E., Hodson, N., Fenlon, M., Zoitopoulos, L., Gallagher, J., 2008. Isolation and identification of *bifidobacteriaceae* from human saliva. Appl Environ Microbiol 74(20), 6457-6460.

Belzer, C., de Vos, W.M., 2012. Microbes inside--from diversity to function: the case of *Akkermansia*. ISME J 6(8), 1449-1458.

Benevides, L., Burman, S., Martin, R., Robert, V., Thomas, M., Miquel, S., Chain, F., Sokol, H., Bermudez-Humaran, L.G., Morrison, M., Langella, P., Azevedo, V.A., Chatel, J.M., Soares, S., 2017. New insights into the diversity of the genus *Faecalibacterium*. Front Microbiol 8, 1790.

Boesmans, L., Valles-Colomer, M., Wang, J., Eeckhaut, V., Falony, G., Ducatelle, R., Van Immerseel, F., Raes, J., Verbeke, K., 2018. Butyrate producers as potential next-generation probiotics: safety assessment of the administration of *Butyricicoccus pullicaecorum* to healthy volunteers. mSystems 3(6), e00094-00018.

Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S.D., Sorokin, A., 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. Genome Res 11(5), 731-753.

Bomba, A., Nemcová, R.r., Mudroňová, D., Guba, P., 2002. The possibilities of potentiating the efficacy of probiotics. Trends Food Sci Technol 13(4), 121-126.

Brodmann, T., Endo, A., Gueimonde, M., Vinderola, G., Kneifel, W., de Vos, W.M., Salminen, S., Gómez-Gallego, C., 2017. Safety of novel microbes for human consumption: practical examples of assessment in the European Union. Front Microbiol 8, 1725.

Broeckx, G., Vandenheuvel, D., Claes, I.J., Lebeer, S., Kiekens, F., 2016. Drying techniques of probiotic bacteria as an important step towards the development of novel pharmabiotics. Int J Pharm 505(1-2), 303-318.

ACCEPTED MANUSCRIPT

Bron, P.A., Marcelli, B., Mulder, J., van der Els, S., Morawska, L.P., Kuipers, O.P., Kok, J., Kleerebezem, M., 2018. Renaissance of traditional DNA transfer strategies for improvement of industrial lactic acid bacteria. Curr Opin Biotechnol 56, 61-68.
Cai, R., Jiang, Y., Yang, W., Yang, W., Shi, S., Shi, C., Hu, J., Gu, W., Ye, L., Zhou, F., Gong, Q., Han, W., Yang, G., Wang, C., 2016. Surface-displayed IL-10 by recombinant *Lactobacillus plantarum* reduces Th1 responses of RAW264.7 cells stimulated with Poly(I:C) or LPS. J Microbiol Biotechnol 26(2), 421-431.
Callanan, M., Kaleta, P., O'Callaghan, J., O'Sullivan, O., Jordan, K., McAuliffe, O., Sangrador-Vegas, A., Slattery, L., Fitzgerald, G.F., Beresford, T., Ross, R.P., 2008. Genome sequence of *Lactobacillus helveticus*, an organism distinguished by selective gene loss and insertion sequence element expansion. J Bacteriol 190(2), 727-735.
Callaway, E., 2018. CRISPR plants now subject to tough GM laws in European Union. Nature 560(7716), 16.
Campedelli, I., Mathur, H., Salvetti, E., Clarke, S., Rea, M.C., Torriani, S., Ross, R.P., Hill, C., O'Toole, P.W., 2018. Genus-wide assessment of antibiotic resistance in *Lactobacillus* spp. Appl Environ Microbiol, pii: AEM.01738-01718.
Cani, P.D., de Vos, W.M., 2017. Next-generation beneficial microbes: the case of *Akkermansia muciniphila*. Front Microbiol 8, 1765.
Cassir, N., Benamar, S., La Scola, B., 2016. *Clostridium butyricum*: from beneficial to a new emerging pathogen. Clin Microbiol Infect 22(1), 37-45.
Ceapa, C., Davids, M., Ritari, J., Lambert, J., Wels, M., Douillard, F.P., Smokvina, T., de Vos, W.M., Knol, J., Kleerebezem, M., 2016. The variable regions of *Lactobacillus rhamnosus* genomes reveal the dynamic evolution of metabolic and host-adaptation repertoires. Genome Biol Evol 8(6), 1889-1905.
Chaillou, S., Lucquin, I., Najjari, A., Zagorec, M., Champomier-Verges, M.C., 2013. Population genetics of *Lactobacillus sakei* reveals three lineages with distinct evolutionary histories. PLoS One 8(9), e73253.
Chassard, C., Delmas, E., Lawson, P.A., Bernalier-Donadille, A., 2008. *Bacteroides xylanisolvens* sp. nov., a xylan-degrading bacterium isolated from human faeces. Int J Syst Evol Microbiol 58(Pt 4), 1008-1013.
Chiron, C., Tompkins, T.A., Burguiere, P., 2018. Flow cytometry: a versatile technology for specific quantification and viability assessment of micro-organisms in multistrain probiotic products. J Appl Microbiol 124(2), 572-584.
Claesson, M.J., Li, Y., Leahy, S., Canchaya, C., van Pijkeren, J.P., Cerdeno-Tarraga, A.M., Parkhill, J., Flynn, S., O'Sullivan, G.C., Collins, J.K., Higgins, D., Shanahan, F., Fitzgerald, G.F., van Sinderen, D., O'Toole, P.W., 2006. Multireplicon genome architecture of *Lactobacillus salivarius*. Proc Natl Acad Sci U S A 103(17), 6718-6723.
Coghetto, C.C., Brinques, G.B., Ayub, M.A., 2016. Probiotics production and alternative encapsulation methodologies to improve their viabilities under adverse environmental conditions. Int J Food Sci Nutr 67(8), 929-943.
Collins, S.M., Surette, M., Bercik, P., 2012. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol 10(11), 735-742.
Commission 2014/907/EU, Commission implementing decision of 11 December 2014 authorising the placing on the market of *Clostridium butyricum* (CBM 588) as a novel food ingredient under regulation (EC) No 258/97 of the European Parliament and of the Council (notified under document C(2014) 9345). in: Commission, E. (Ed.).
Cotillard, A., Kennedy, S.P., Kong, L.C., Prifti, E., Pons, N., Le Chatelier, E., Almeida, M., Quinquis, B., Levenez, F., Galleron, N., Gougis, S., Rizkalla, S., Batto, J.M., Renault, P., consortium, A.N.R.M., Dore, J., Zucker, J.D., Clement, K., Ehrlich, S.D., 2013. Dietary intervention impact on gut microbial gene richness. Nature 500(7464), 585-588.
de Melo Pereira, G.V., de Oliveira Coelho, B., Magalhaes Junior, A.I., Thomaz-Soccol, V., Soccol, C.R., 2018. How to select a probiotic? A review and update of methods and criteria. Biotechnol Adv(36(8)), 2060-2076.
Derkx, P.M., Janzen, T., Sorensen, K.I., Christensen, J.E., Stuer-Lauridsen, B., Johansen, E., 2014. The art of strain improvement of industrial lactic acid bacteria without the use of recombinant DNA technology. Microb Cell Fact 13 Suppl 1, S5.
Derrien, M., Belzer, C., de Vos, W.M., 2017. *Akkermansia muciniphil*a and its role in regulating host functions. Microb Pathog 106, 171-181.

ACCEPTED MANUSCRIPT

Derrien, M., Van Baarlen, P., Hooiveld, G., Norin, E., Muller, M., de Vos, W.M., 2011. Modulation of mucosal immune response, tolerance, and proliferation in mice colonized by the mucin-degrader *Akkermansia muciniphila*. Front Microbiol 2, 166.
Derrien, M., Vaughan, E.E., Plugge, C.M., de Vos, W.M., 2004. *Akkermansia muciniphila* gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 54(Pt 5), 1469-1476.
Directive 2001/18/EC, in: Union, C.D.E.P.a.C.o.t.E. (Ed.) Off. J. Eur. Commun. pp. 1–38.
Douglas, G.L., Klaenhammer, T.R., 2010. Genomic evolution of domesticated microorganisms. Annu Rev Food Sci Technol 1, 397-414.
Douillard, F.P., Ribbera, A., Kant, R., Pietila, T.E., Jarvinen, H.M., Messing, M., Randazzo, C.L., Paulin, L., Laine, P., Ritari, J., Caggia, C., Lahteinen, T., Brouns, S.J., Satokari, R., von Ossowski, I., Reunanen, J., Palva, A., de Vos, W.M., 2013. Comparative genomic and functional analysis of 100 *Lactobacillus rhamnosus* strains and their comparison with strain GG. PLoS Genet 9(8), e1003683.
Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol 70(10), 5810-5817.
Eeckhaut, V., Ducatelle, R., Sas, B., Vermeire, S., Van Immerseel, F., 2014. Progress towards butyrate-producing pharmabiotics: *Butyricicoccus pullicaecorum* capsule and efficacy in TNBS models in comparison with therapeutics. Gut 63(2), 367.
Eeckhaut, V., Machiels, K., Perrier, C., Romero, C., Maes, S., Flahou, B., Steppe, M., Haesebrouck, F., Sas, B., Ducatelle, R., Vermeire, S., Van Immerseel, F., 2013. *Butyricicoccus pullicaecorum* in inflammatory bowel disease. Gut 62(12), 1745-1752.
Eeckhaut, V., Van Immerseel, F., Teirlynck, E., Pasmans, F., Fievez, V., Snauwaert, C., Haesebrouck, F., Ducatelle, R., Louis, P., Vandamme, P., 2008. *Butyricicoccus pullicaecorum* gen. nov., sp. nov., an anaerobic, butyrate-producing bacterium isolated from the caecal content of a broiler chicken. Int J Syst Evol Microbiol 58(Pt 12), 2799-2802.
EFSA NDA Panel, 2015. Scientific opinion on the safety of 'heat-treated milk products fermented with *Bacteroides xylanisolvens* DSM 23964' as a novel food. EFSA, p. 3956.
El-Semman, I.E., Karlsson, F.H., Shoaie, S., Nookaew, I., Soliman, T.H., Nielsen, J., 2014. Genome-scale metabolic reconstructions of *Bifidobacterium adolescentis* L2-32 and *Faecalibacterium prausnitzii* A2-165 and their interaction. BMC Syst Biol 8, 41.
El Hage, R., Hernandez-Sanabria, E., Van de Wiele, T., 2017. Emerging trends in "Smart Probiotics": functional consideration for the development of novel health and industrial applications. Front Microbiol 8, 1889.
Engels, C., Ruscheweyh, H.J., Beerenwinkel, N., Lacroix, C., Schwab, C., 2016. The common gut microbe *Eubacterium hallii* also contributes to intestinal propionate formation. Front Microbiol 7, 713.
Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J.P., Druart, C., Bindels, L.B., Guiot, Y., Derrien, M., Muccioli, G.G., Delzenne, N.M., de Vos, W.M., Cani, P.D., 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 110(22), 9066-9071.
Falentin, H., Deutsch, S.M., Loux, V., Hammani, A., Buratti, J., Parayre, S., Chuat, V., Barbe, V., Aury, J.M., Jan, G., Le Loir, Y., 2016. Permanent draft genome sequence of the probiotic strain *Propionibacterium freudenreichii* CIRM-BIA 129 (ITG P20). Stand Genomic Sci 11, 6.
Fleischmann, R.D., Adams, M.D., White, O., Clayton, R.A., Kirkness, E.F., Kerlavage, A.R., Bult, C.J., Tomb, J.F., Dougherty, B.A., Merrick, J.M., et al., 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 269(5223), 496-512.
Flemer, B., Warren, R.D., Barrett, M.P., Cisek, K., Das, A., Jeffery, I.B., Hurley, E., O'Riordain, M., Shanahan, F., O'Toole, P.W., 2018. The oral microbiota in colorectal cancer is distinctive and predictive. Gut 67(8), 1454-1463.
Fu, N., Huang, S., Xiao, J., Chen, X.D., 2018. Producing powders containing active dry probiotics with the aid of spray drying. Adv Food Nutr Res 85, 211-262.
Garrigues, C., Johansen, E., Pedersen, M.B., 2010. Complete genome sequence of *Bifidobacterium animalis* subsp. *lactis* BB-12, a widely consumed probiotic strain. J Bacteriol 192(9), 2467-2468.
Geerlings, S.Y., Kostopoulos, I., de Vos, W.M., Belzer, C., 2018. *Akkermansia muciniphila* in the human gastrointestinal tract: when, where, and how? Microorganisms 6(3).

ACCEPTED MANUSCRIPT

Gilbert, J.A., Quinn, R.A., Debelius, J., Xu, Z.Z., Morton, J., Garg, N., Jansson, J.K., Dorrestein, P.C., Knight, R., 2016. Microbiome-wide association studies link dynamic microbial consortia to disease. Nature 535(7610), 94-103.
Global Market Insight, 2018. Probiotics market growth - Industry size, share research 2018-2024. Global Market Insight.
Goldin, B.R., Gorbach, S.L., Saxelin, M., Barakat, S., Gualtieri, L., Salminen, S., 1992. Survival of *Lactobacillus* species (strain GG) in human gastrointestinal tract. Dig Dis Sci 37(1), 121-128.
Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J.T., Spector, T.D., Clark, A.G., Ley, R.E., 2014. Human genetics shape the gut microbiome. Cell 159(4), 789-799.
Gueimonde, M., Sanchez, B., 2012. Enhancing probiotic stability in industrial processes. Microb Ecol Health Dis 23.
Harris, H.M.B., Bourin, M.J.B., Claesson, M.J., O'Toole, P.W., 2017. Phylogenomics and comparative genomics of *Lactobacillus salivarius*, a mammalian gut commensal. Microb Genom 3(8), e000115.
Hemarajata, P., Versalovic, J., 2013. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol 6(1), 39-51.
Henderson, G., Ulsemer, P., Schober, U., Loffler, A., Alpert, C.A., Zimmermann-Kordmann, M., Reutter, W., Karsten, U., Goletz, S., Blaut, M., 2011. Occurrence of the human tumor-specific antigen structure Galbeta1-3GalNAcalpha- (Thomsen-Friedenreich) and related structures on gut bacteria: prevalence, immunochemical analysis and structural confirmation. Glycobiology 21(10), 1277-1289.
Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., Sanders, M.E., 2014. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11(8), 506-514.
Hugenholtz, F., de Vos, W.M., 2018. Mouse models for human intestinal microbiota research: a critical evaluation. Cell Mol Life Sci 75(1), 149-160.
Kahrstrom, C.T., Pariente, N., Weiss, U., 2016. Intestinal microbiota in health and disease. Nature 535(7610), 47.
Kajander, K., Myllyluoma, E., Rajilic-Stojanovic, M., Kyronpalo, S., Rasmussen, M., Jarvenpaa, S., Zoetendal, E.G., de Vos, W.M., Vapaatalo, H., Korpela, R., 2008. Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther 27(1), 48-57.
Kankainen, M., Paulin, L., Tynkkynen, S., von Ossowski, I., Reunanen, J., Partanen, P., Satokari, R., Vesterlund, S., Hendrickx, A.P., Lebeer, S., De Keersmaecker, S.C., Vanderleyden, J., Hämäläinen, T., Laukkanen, S., Salovuori, N., Ritari, J., Alatalo, E., Korpela, R., Mattila-Sandholm, T., Lassig, A., Hatakka, K., Kinnunen, K.T., Karjalainen, H., Saxelin, M., Laakso, K., Surakka, A., Palva, A., Salusjärvi, T., Auvinen, P., de Vos, W.M., 2009. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human- mucus binding protein. Proc Natl Acad Sci U S A 106(40), 17193-17198.
Karimi, O., Pena, A.S., 2008. Indications and challenges of probiotics, prebiotics, and synbiotics in the management of arthralgias and spondyloarthropathies in inflammatory bowel disease. J Clin Gastroenterol 42 Suppl 3 Pt 1, S136-S141.
Khalili, L., Alipour, B., Asghari Jafar-Abadi, M., Faraji, I., Hassanalilou, T., Mesgari Abbasi, M., Vaghef-Mehrabany, E., Alizadeh Sani, M., 2018. The effects of *Lactobacillus casei* on glycemic response, serum Sirtuin1 and Fetuin-A levels in patients with type 2 diabetes mellitus: a randomized controlled trial. Iran Biomed J 23(1), 68-77.
Khan, M.T., van Dijl, J.M., Harmsen, H.J., 2014. Antioxidants keep the potentially probiotic but highly oxygen-sensitive human gut bacterium *Faecalibacterium prausnitzii* alive at ambient air. PLoS One 9(5), e96097.
Kim, S., Huang, E., Park, S., Holzapfel, W., Lim, S.D., 2018. Physiological characteristics and anti-obesity effect of *Lactobacillus plantarum* K10. Korean J Food Sci Anim Resour 38(3), 554-569.

ACCEPTED MANUSCRIPT

Kleerebezem, M., Binda, S., Bron, P.A., Gross, G., Hill, C., van Hylckama Vlieg, J.E., Lebeer, S., Satokari, R., Ouwehand, A.C., 2018. Understanding mode of action can drive the translational pipeline towards more reliable health benefits for probiotics. Curr Opin Biotechnol 56, 55-60.
Koo, O.K., Amalaradjou, M.A., Bhunia, A.K., 2012. Recombinant probiotic expressing *Listeria* adhesion protein attenuates *Listeria monocytogenes* virulence *in vitro*. PLoS One 7(1), e29277.
Koponen, J., Laakso, K., Koskenniemi, K., Kankainen, M., Savijoki, K., Nyman, T.A., de Vos, W.M., Tynkkynen, S., Kalkkinen, N., Varmanen, P., 2012. Effect of acid stress on protein expression and phosphorylation in *Lactobacillus rhamnosus* GG. J Proteomics 75(4), 1357-1374.
Lebeer, S., Bron, P.A., Marco, M.L., Van Pijkeren, J.P., O'Connell Motherway, M., Hill, C., Pot, B., Roos, S., Klaenhammer, T., 2018. Identification of probiotic effector molecules: present state and future perspectives. Curr Opin Biotechnol 49, 217-223.
Lebeer, S., Vanderleyden, J., De Keersmaecker, S.C., 2008. Genes and molecules of lactobacilli supporting probiotic action. Microbiol Mol Biol Rev 72(4), 728-764, Table of Contents.
Lee, E., Jung, S.R., Lee, S.Y., Lee, N.K., Paik, H.D., Lim, S.I., 2018. *Lactobacillus plantarum s*train Ln4 attenuates diet-induced obesity, insulin resistance, and changes in hepatic mRNA levels associated with glucose and lipid metabolism. Nutrients 10(5).
Lee, S., You, H.J., Kwon, B., Ko, G., 2017. Complete genome sequence of *Lactobacillus jensenii* strain SNUV360, a probiotic for treatment of bacterial vaginosis isolated from the vagina of a healthy Korean woman. Genome Announc 5(10).
Leenay, R.T., Vento, J.M., Shah, M., Martino, M.E., Leulier, F., Beisel, C.L., 2018. Genome editing with CRISPR-Cas9 in *Lactobacillus plantarum* revealed that editing outcomes can vary across strains and between methods. Biotechnol J, e1700583.
Liu, W.C., Yang, M.C., Wu, Y.Y., Chen, P.H., Hsu, C.M., Chen, L.W., 2018. *Lactobacillus plantarum* reverse diabetes-induced Fmo3 and ICAM expression in mice through enteric dysbiosis-related c-Jun NH2-terminal kinase pathways. PLoS One 13(5), e0196511.
Lopez-Siles, M., Duncan, S.H., Garcia-Gil, L.J., Martinez-Medina, M., 2017. *Faecalibacterium prausnitzii*: from microbiology to diagnostics and prognostics. ISME J 11(4), 841-852.
Maier, E., Anderson, R.C., Roy, N.C., 2017. Live *Faecalibacterium prausnitzii* does not enhance epithelial barrier integrity in an apical anaerobic co-culture model of the large intestine. Nutrients 9(12), E1349.
Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N., Karamychev, V., Polouchine, N., Shakhova, V., Grigoriev, I., Lou, Y., Rohksar, D., Lucas, S., Huang, K., Goodstein, D.M., Hawkins, T., Plengvidhya, V., Welker, D., Hughes, J., Goh, Y., Benson, A., Baldwin, K., Lee, J.H., Diaz-Muniz, I., Dosti, B., Smeianov, V., Wechter, W., Barabote, R., Lorca, G., Altermann, E., Barrangou, R., Ganesan, B., Xie, Y., Rawsthorne, H., Tamir, D., Parker, C., Breidt, F., Broadbent, J., Hutkins, R., O'Sullivan, D., Steele, J., Unlu, G., Saier, M., Klaenhammer, T., Richardson, P., Kozyavkin, S., Weimer, B., Mills, D., 2006. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci U S A 103(42), 15611-15616.
Mangiapane, E., Mazzoli, R., Pessione, A., Svensson, B., Riedel, K., Pessione, E., 2015. Ten years of subproteome investigations in lactic acid bacteria: a key for food starter and probiotic typing. J Proteomics 127(Pt B), 332-339.
Marcial-Coba, M.S., Cieplak, T., Cahu, T.B., Blennow, A., Knochel, S., Nielsen, D.S., 2018. Viability of microencapsulated *Akkermansia muciniphila* and *Lactobacillus plantarum* during freeze-drying, storage and *in vitro* simulated upper gastrointestinal tract passage. Food Funct 9(11), 5868-5879.
Markets and Markets, 2017. Human Microbiome Market– _Global Forecast to 2023. Markets and Markets.
Martin, R., Bermudez-Humaran, L.G., Langella, P., 2018. Searching for the bacterial effector: the example of the multi-skilled commensal bacterium *Faecalibacterium prausnitzii*. Front Microbiol 9, 346.
Martin, R., Miquel, S., Benevides, L., Bridonneau, C., Robert, V., Hudault, S., Chain, F., Berteau, O., Azevedo, V., Chatel, J.M., Sokol, H., Bermudez-Humaran, L.G., Thomas, M., Langella, P., 2017. Functional characterization of novel F*aecalibacterium prausnitzii* strains isolated from healthy volunteers: a step forward in the use of *F. prausnitzii* as a next-generation probiotic. Front Microbiol 8, 1226.
Mathipa, M.G., Thantsha, M.S., 2017. Probiotic engineering: towards development of robust probiotic strains with enhanced functional properties and for targeted control of enteric pathogens. Gut Pathog 9, 28.

ACCEPTED MANUSCRIPT

Mays, Z.J., Nair, N.U., 2018. Synthetic biology in probiotic lactic acid bacteria: At the frontier of living therapeutics. Curr Opin Biotechnol 53, 224-231.
Maze, A., Boel, G., Zuniga, M., Bourand, A., Loux, V., Yebra, M.J., Monedero, V., Correia, K., Jacques, N., Beaufils, S., Poncet, S., Joyet, P., Milohanic, E., Casaregola, S., Auffray, Y., Perez-Martinez, G., Gibrat, J.F., Zagorec, M., Francke, C., Hartke, A., Deutscher, J., 2010. Complete genome sequence of the probiotic *Lactobacillus casei* strain BL23. J Bacteriol 192(10), 2647-2648.
McKellar, S., Tzafestas, K., Brayne, S., 2017. Exploiting the microbiome . Market developments andintellectual property landscape. IP Pragmatics Ltd.
Michon, C., Langella, P., Eijsink, V.G., Mathiesen, G., Chatel, J.M., 2016. Display of recombinant proteins at the surface of lactic acid bacteria: strategies and applications. Microb Cell Fact 15, 70.
Milani, C., Duranti, S., Lugli, G.A., Bottacini, F., Strati, F., Arioli, S., Foroni, E., Turroni, F., van Sinderen, D., Ventura, M., 2013. Comparative genomics of *Bifidobacterium animalis* subsp. *lactis* reveals a strict monophyletic bifidobacterial taxon. Appl Environ Microbiol 79(14), 4304-4315.
Miquel, S., Martin, R., Rossi, O., Bermudez-Humaran, L.G., Chatel, J.M., Sokol, H., Thomas, M., Wells, J.M., Langella, P., 2013. *Faecalibacterium prausnitzii* and human intestinal health. Curr Opin Microbiol 16(3), 255-261.
Mo, S., Kim, B.S., Yun, S.J., Lee, J.J., Yoon, S.H., Oh, C.H., 2015. Genome sequencing of *Clostridium butyricum* DKU-01, isolated from infant feces. Gut Pathog 7, 8.
Motta, J.P., Bermudez-Humaran, L.G., Deraison, C., Martin, L., Rolland, C., Rousset, P., Boue, J., Dietrich, G., Chapman, K., Kharrat, P., Vinel, J.P., Alric, L., Mas, E., Sallenave, J.M., Langella, P., Vergnolle, N., 2012. Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis. Sci Transl Med 4(158), 158ra144.
Mougiakos, I., Bosma, E.F., de Vos, W.M., van Kranenburg, R., van der Oost, J., 2016. Next generation prokaryotic engineering: the CRISPR-Cas toolkit. Trends Biotechnol 34(7), 575-587.
Nakanishi, S., Tanaka, M., 2010. Sequence analysis of a bacteriocinogenic plasmid of *Clostridium butyricum* and expression of the bacteriocin gene in *Escherichia coli*. Anaerobe 16(3), 253-257.
O'Connell Motherway, M., Zomer, A., Leahy, S.C., Reunanen, J., Bottacini, F., Claesson, M.J., O'Brien, F., Flynn, K., Casey, P.G., Munoz, J.A., Kearney, B., Houston, A.M., O'Mahony, C., Higgins, D.G., Shanahan, F., Palva, A., de Vos, W.M., Fitzgerald, G.F., Ventura, M., O'Toole, P.W., van Sinderen, D., 2011. Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. Proc Natl Acad Sci U S A 108(27), 11217-11222.
O'Toole, P.W., Marchesi, J.R., Hill, C., 2017. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. Nat Microbiol 2, 17057.
Occams Business, 2017. Global probiotic market-technologies, market share and industry forecast to 2024. Occams Business Research & Consulting Pvt. Ltd.
Oka, K., Osaki, T., Hanawa, T., Kurata, S., Sugiyama, E., Takahashi, M., Tanaka, M., Taguchi, H., Kamiya, S., 2018. Establishment of an endogenous *Clostridium difficile* rat infection model and evaluation of the effects of *Clostridium butyricum* MIYAIRI 588 probiotic strain. Front Microbiol 9, 1264.
Ottman, N., Huuskonen, L., Reunanen, J., Boeren, S., Klievink, J., Smidt, H., Belzer, C., de Vos, W.M., 2016. Characterization of outer membrane proteome of *Akkermansia muciniphila* reveals sets of novel proteins exposed to the human intestine. Front Microbiol 7, 1157.
Park, S.S., Lee, Y.J., Song, S., Kim, B., Kang, H., Oh, S., Kim, E., 2018. *Lactobacillus acidophilus* NS1 attenuates diet-induced obesity and fatty liver. J Endocrinol 237(2), 87-100.
Piwowarek, K., Lipinska, E., Hac-Szymanczuk, E., Kieliszek, M., Scibisz, I., 2018. *Propionibacterium* spp.-source of propionic acid, vitamin B12, and other metabolites important for the industry. Appl Microbiol Biotechnol 102(2), 515-538.
Plovier, H., Everard, A., Druart, C., Depommier, C., Van Hul, M., Geurts, L., Chilloux, J., Ottman, N., Duparc, T., Lichtenstein, L., Myridakis, A., Delzenne, N.M., Klievink, J., Bhattacharjee, A., van der Ark, K.C., Aalvink, S., Martinez, L.O., Dumas, M.E., Maiter, D., Loumaye, A., Hermans, M.P., Thissen, J.P., Belzer, C., de Vos, W.M., Cani, P.D., 2017. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med 23(1), 107-113.

ACCEPTED MANUSCRIPT

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D.R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J.M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H.B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Dore, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J., Meta, H.I.T.C., Bork, P., Ehrlich, S.D., Wang, J., 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464(7285), 59-65.

Quevrain, E., Maubert, M.A., Michon, C., Chain, F., Marquant, R., Tailhades, J., Miquel, S., Carlier, L., Bermudez-Humaran, L.G., Pigneur, B., Lequin, O., Kharrat, P., Thomas, G., Rainteau, D., Aubry, C., Breyner, N., Afonso, C., Lavielle, S., Grill, J.P., Chassaing, G., Chatel, J.M., Trugnan, G., Xavier, R., Langella, P., Sokol, H., Seksik, P., 2016. Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. Gut 65(3), 415-425.

Rajilić-Stojanović, M., de Vos, W.M., 2014. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 38(5), 996-1047.

Ramirez, A.M., Rodriguez-Lopez, A., Ardila, A., Beltran, L., Patarroyo, C.A., Melendez, A.D.P., Sanchez, O.F., Almeciga-Diaz, C.J., 2017. Production of human recombinant phenylalanine hydroxylase in Lactobacillus plantarum for gastrointestinal delivery. Eur J Pharm Sci 109, 48-55.

Rasinkangas, P., 2016. New insights into the biogenesis of *Lactobacillus rhamnosus* GG pili and the *in vivo* effects of pili, Faculty of Veterinary Medicine, Department of Veterinary Biosciences. University of Helsinki, Helsinki, p. 88.

Rasinkangas, P., Reunanen, J., Douillard, F.P., Ritari, J., Uotinen, V., Palva, A., de Vos, W.M., 2014. Genomic characterization of non-mucus-adherent derivatives of *Lactobacillus rhamnosus* GG reveals genes affecting pilus biogenesis. Appl Environ Microbiol 80(22), 7001-7009.

Rezzonico, E., Lariani, S., Barretto, C., Cuanoud, G., Giliberti, G., Delley, M., Arigoni, F., Pessi, G., 2007. Global transcriptome analysis of the heat shock response of *Bifidobacterium longum*. FEMS Microbiol Lett 271(1), 136-145.

Riboulet-Bisson, E., Sturme, M.H., Jeffery, I.B., O'Donnell, M.M., Neville, B.A., Forde, B.M., Claesson, M.J., Harris, H., Gardiner, G.E., Casey, P.G., Lawlor, P.G., O'Toole, P.W., Ross, R.P., 2012. Effect of *Lactobacillus salivarius* bacteriocin Abp118 on the mouse and pig intestinal microbiota. PLoS One 7(2), e31113.

Robert, S., Steidler, L., 2014. Recombinant *Lactococcus lactis* can make the difference in antigen-specific immune tolerance induction, the Type 1 Diabetes case. Microb Cell Fact 13 Suppl 1, S11.

Robinson, O., Dylus, D., Dessimoz, C., 2016. Phylo.io: interactive viewing and comparison of large phylogenetic trees on the web. Mol Biol Evol 33(8), 2163-2166.

Roots Analysis Business Research & Consulting, 2017. Microbiome Therapeutic and Diagnostic Markets Second Edition ed. Roots Analysis Business Research & Consulting

Rosa, B.A., Hallsworth-Pepin, K., Martin, J., Wollam, A., Mitreva, M., 2017. Genome Sequence of *Christensenella minuta* DSM 22607^{T}. Genome Announc 5(2), e01451-01416.

Rossi, O., van Berkel, L.A., Chain, F., Tanweer Khan, M., Taverne, N., Sokol, H., Duncan, S.H., Flint, H.J., Harmsen, H.J., Langella, P., Samsom, J.N., Wells, J.M., 2016. *Faecalibacterium prausnitzii* A2-165 has a high capacity to induce IL-10 in human and murine dendritic cells and modulates T cell responses. Sci Rep 6, 18507.

Routy, B., Le Chatelier, E., Derosa, L., Duong, C.P.M., Alou, M.T., Daillere, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M.P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragon, L., Jacquelot, N., Qu, B., Ferrere, G., Clemenson, C., Mezquita, L., Masip, J.R., Naltet, C., Brosseau, S., Kaderbhai, C., Richard, C., Rizvi, H., Levenez, F., Galleron, N., Quinquis, B., Pons, N., Ryffel, B., Minard-Colin, V., Gonin, P., Soria, J.C., Deutsch, E., Loriot, Y., Ghiringhelli, F., Zalcman, G., Goldwasser, F., Escudier, B., Hellmann, M.D., Eggermont, A., Raoult, D., Albiges, L., Kroemer, G., Zitvogel, L., 2018. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 359(6371), 91-97.

Rudzki, L., Ostrowska, L., Pawlak, D., Malus, A., Pawlak, K., Waszkiewicz, N., Szulc, A., 2018. Probiotic *Lactobacillus plantarum* 299v decreases kynurenine concentration and improves cognitive functions in

ACCEPTED MANUSCRIPT

patients with major depression: A double-blind, randomized, placebo controlled study. Psychoneuroendocrinology 100, 213-222.
Ruiz, L., Zomer, A., O'Connell-Motherway, M., van Sinderen, D., Margolles, A., 2012. Discovering novel bile protection systems in *Bifidobacterium breve* UCC2003 through functional genomics. Appl Environ Microbiol 78(4), 1123-1131.
Salonen, A., de Vos, W.M., 2014. Impact of diet on human intestinal microbiota and health. Annu Rev Food Sci Technol 5, 239-262.
Salvetti, E., Harris, H.M.B., Felis, G.E., O'Toole, P.W., 2018. Comparative genomics of the genus *Lactobacillus* reveals robust phylogroups that provide the basis for reclassification. Appl Environ Microbiol 84(17), e00993-00918.
Sanders, M.E., Benson, A., Lebeer, S., Merenstein, D.J., Klaenhammer, T.R., 2018. Shared mechanisms among probiotic taxa: implications for general probiotic claims. Curr Opin Biotechnol 49, 207-216.
Schroeder, B.O., Backhed, F., 2016. Signals from the gut microbiota to distant organs in physiology and disease. Nat Med 22(10), 1079-1089.
Seki, H., Shiohara, M., Matsumura, T., Miyagawa, N., Tanaka, M., Komiyama, A., Kurata, S., 2003. Prevention of antibiotic-associated diarrhea in children by *Clostridium butyricum* MIYAIRI. Pediatr Int 45(1), 86-90.
Seme, H., Bogovic Matijasic, B., Svigelj, K., Langerholc, T., Fujs, S., Horvat, J., Zlatic, E., Gjuracic, K., Petkovic, H., Stempelj, M., Kos, B., Suskovic, J., Kosec, G., 2017. Generation of *Lactobacillus plantarum* strains with improved potential to target gastrointestinal disorders related to sugar malabsorption. Food Res Int 94, 45-53.
Sheehan, V.M., Sleator, R.D., Fitzgerald, G.F., Hill, C., 2006. Heterologous expression of BetL, a betaine uptake system, enhances the stress tolerance of *Lactobacillus salivarius* UCC118. Appl Environ Microbiol 72(3), 2170-2177.
Sherwin, E., Rea, K., Dinan, T.G., Cryan, J.F., 2016. A gut (microbiome) feeling about the brain. Curr Opin Gastroenterol 32(2), 96-102.
Shetty, S.A., 2019. Ecophysiological insights into the human intestinal microbiota: from single strains to defined consortia., Microbiology. Wageningen University.
Shetty, S.A., Ritari, J., Paulin, L., Smidt, H., De Vos, W.M., 2017. Complete genome sequence of *Eubacterium hallii* strain L2-7. Genome Announc 5(43), e01167-01117.
Shetty, S.A., Zuffa, S., Bui, T.P.N., Aalvink, S., Smidt, H., De Vos, W.M., 2018. Reclassification of *Eubacterium hallii* as *Anaerobutyricum hallii* gen. nov., comb. nov., and description of *Anaerobutyricum soehngenii* sp. nov., a butyrate and propionate-producing bacterium from infant faeces. Int J Syst Evol Microbiol.
Shortt, C., 1999. The probiotic century: historical and current perspectives. Trends in Food Science & Technology 10(12), 411-417.
Siezen, R.J., Francke, C., Renckens, B., Boekhorst, J., Wels, M., Kleerebezem, M., van Hijum, S.A., 2012. Complete resequencing and reannotation of the *Lactobacillus plantarum* WCFS1 genome. J Bacteriol 194(1), 195-196.
Sipailiene, A., Petraityte, S., 2018. Encapsulation of probiotics: proper selection of the probiotic strain and the influence of encapsulation technology and materials on the viability of encapsulated microorganisms. Probiotics Antimicrob Proteins 10(1), 1-10.
Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L.G., Gratadoux, J.J., Blugeon, S., Bridonneau, C., Furet, J.P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottiere, H.M., Dore, J., Marteau, P., Seksik, P., Langella, P., 2008. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 105(43), 16731-16736.
Sonnenburg, J.L., Backhed, F., 2016. Diet-microbiota interactions as moderators of human metabolism. Nature 535(7610), 56-64.
Sornplang, P., Piyadeatsoontorn, S., 2016. Probiotic isolates from unconventional sources: a review. J Anim Sci Technol 58, 26.
Steppe, M., Van Nieuwerburgh, F., Vercauteren, G., Boyen, F., Eeckhaut, V., Deforce, D., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2014. Safety assessment of the butyrate-producing *Butyricicoccus*

ACCEPTED MANUSCRIPT

pullicaecorum strain 25-3(T), a potential probiotic for patients with inflammatory bowel disease, based on oral toxicity tests and whole genome sequencing. Food Chem Toxicol 72, 129-137.
Sun, Z., Harris, H.M., McCann, A., Guo, C., Argimon, S., Zhang, W., Yang, X., Jeffery, I.B., Cooney, J.C., Kagawa, T.F., Liu, W., Song, Y., Salvetti, E., Wrobel, A., Rasinkangas, P., Parkhill, J., Rea, M.C., O'Sullivan, O., Ritari, J., Douillard, F.P., Paul Ross, R., Yang, R., Briner, A.E., Felis, G.E., de Vos, W.M., Barrangou, R., Klaenhammer, T.R., Caufield, P.W., Cui, Y., Zhang, H., O'Toole, P.W., 2015. Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. Nat Commun 6, 8322.
Szatraj, K., Szczepankowska, A.K., Chmielewska-Jeznach, M., 2017. Lactic acid bacteria - promising vaccine vectors: possibilities, limitations, doubts. J Appl Microbiol 123(2), 325-339.
Taroncher-Oldenburg, G., Jones, S., Blaser, M., Bonneau, R., Christey, P., Clemente, J.C., Elinav, E., Ghedin, E., Huttenhower, C., Kelly, D., Kyle, D., Littman, D., Maiti, A., Maue, A., Olle, B., Segal, L., van Hylckama Vlieg, J.E.T., Wang, J., 2018. Translating microbiome futures. Nat Biotechnol 36(11), 1037-1042.
Teusink, B., Wiersma, A., Molenaar, D., Francke, C., de Vos, W.M., Siezen, R.J., Smid, E.J., 2006. Analysis of growth of *Lactobacillus plantarum* WCFS1 on a complex medium using a genome-scale metabolic model. J Biol Chem 281(52), 40041-40048.
Thaiss, C.A., Zmora, N., Levy, M., Elinav, E., 2016. The microbiome and innate immunity. Nature 535(7610), 65-74.
Torriani, S., Felis, G.E., Dellaglio, F., 2001. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with recA gene-derived primers. Appl Environ Microbiol 67(8), 3450-3454.
Udayappan, S., Manneras-Holm, L., Chaplin-Scott, A., Belzer, C., Herrema, H., Dallinga-Thie, G.M., Duncan, S.H., Stroes, E.S.G., Groen, A.K., Flint, H.J., Backhed, F., de Vos, W.M., Nieuwdorp, M., 2016. Oral treatment with *Eubacterium hallii* improves insulin sensitivity in db/db mice. NPJ Biofilms Microbiomes 2, 16009.
Ulsemer, P., Toutounian, K., Kressel, G., Goletz, C., Schmidt, J., Karsten, U., Hahn, A., Goletz, S., 2016. Impact of oral consumption of heat-treated *Bacteroides xylanisolvens* DSM 23964 on the level of natural TF-alpha-specific antibodies in human adults. Benef Microbes 7(4), 485-500.
van der Ark, K.C.H., Aalvink, S., Suarez-Diez, M., Schaap, P.J., de Vos, W.M., Belzer, C., 2018. Model-driven design of a minimal medium for *Akkermansia muciniphila* confirms mucus adaptation. Microb Biotechnol 11(3), 476-485.
van der Ark, K.C.H., Nugroho, A.D.W., Berton-Carabin, C., Wang, C., Belzer, C., de Vos, W.M., Schroen, K., 2017a. Encapsulation of the therapeutic microbe *Akkermansia muciniphila* in a double emulsion enhances survival in simulated gastric conditions. Food Res Int 102, 372-379.
van der Ark, K.C.H., van Heck, R.G.A., Martins Dos Santos, V.A.P., Belzer, C., de Vos, W.M., 2017b. More than just a gut feeling: constraint-based genome-scale metabolic models for predicting functions of human intestinal microbes. Microbiome 5(1), 78.
van Passel, M.W., Kant, R., Zoetendal, E.G., Plugge, C.M., Derrien, M., Malfatti, S.A., Chain, P.S., Woyke, T., Palva, A., de Vos, W.M., Smidt, H., 2011. The genome of *Akkermansia muciniphila*, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One 6(3), e16876.
van Pijkeren, J.P., Barrangou, R., 2017. Genome editing of food-grade lactobacilli to develop therapeutic probiotics. Microbiol Spectr 5(5), BAD-0013-2016.
van Pijkeren, J.P., Canchaya, C., Ryan, K.A., Li, Y., Claesson, M.J., Sheil, B., Steidler, L., O'Mahony, L., Fitzgerald, G.F., van Sinderen, D., O'Toole, P.W., 2006. Comparative and functional analysis of sortase-dependent proteins in the predicted secretome of *Lactobacillus salivarius* UCC118. Appl Environ Microbiol 72(6), 4143-4153.
Wang, S., Zhu, H., He, F., Luo, Y., Kang, Z., Lu, C., Feng, L., Lu, X., Xue, Y., Wang, H., 2014. Whole genome sequence of the probiotic strain *Lactobacillus paracasei* N1115, isolated from traditional Chinese fermented milk. Genome Announc 2(2), e00059-00014.
Wattam, A.R., Davis, J.J., Assaf, R., Boisvert, S., Brettin, T., Bun, C., Conrad, N., Dietrich, E.M., Disz, T., Gabbard, J.L., Gerdes, S., Henry, C.S., Kenyon, R.W., Machi, D., Mao, C., Nordberg, E.K., Olsen, G.J., Murphy-Olson, D.E., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Vonstein, V., Warren, A., Xia, F.,

ACCEPTED MANUSCRIPT

Yoo, H., Stevens, R.L., 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res 45(D1), D535-D542.
Wei, Y.X., Zhang, Z.Y., Liu, C., Zhu, Y.Z., Zhu, Y.Q., Zheng, H., Zhao, G.P., Wang, S., Guo, X.K., 2010. Complete genome sequence of *Bifidobacterium longum* JDM301. J Bacteriol 192(15), 4076-4077.
Vrieze, A., Van Nood, E., Holleman, F., Salojarvi, J., Kootte, R.S., Bartelsman, J.F., Dallinga-Thie, G.M., Ackermans, M.T., Serlie, M.J., Oozeer, R., Derrien, M., Druesne, A., Van Hylckama Vlieg, J.E., Bloks, V.W., Groen, A.K., Heilig, H.G., Zoetendal, E.G., Stroes, E.S., de Vos, W.M., Hoekstra, J.B., Nieuwdorp, M., 2012. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 143(4), 913-916 e917.
Yamane, T., Sakamoto, T., Nakagaki, T., Nakano, Y., 2018. Lactic acid bacteria from kefir increase cytotoxicity of natural killer cells to tumor cells. Foods 7(4), E48.
Yang, Y., Gu, H., Sun, Q., Wang, J., 2018. Effects of *Christensenella minuta* lipopolysaccharide on RAW 264.7 macrophages activation. Microb Pathog 125, 411-417.
Yu, D.S., Jeong, H., Lee, D.H., Kwon, S.K., Song, J.Y., Kim, B.K., Park, M.S., Ji, G.E., Oh, T.K., Kim, J.F., 2012. Complete genome sequence of the probiotic bacterium *Bifidobacterium bifidum* strain BGN4. J Bacteriol 194(17), 4757-4758.
Zhao, Y., Duan, C., Gao, L., Yu, X., Niu, C., Li, S., 2017. Genome shuffling of *Lactobacillus plantarum* C88 improves adhesion. Biosci Biotechnol Biochem 81(1), 184-193.
Zoetendal, E.G., Raes, J., van den Bogert, B., Arumugam, M., Booijink, C.C., Troost, F.J., Bork, P., Wels, M., de Vos, W.M., Kleerebezem, M., 2012. The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. ISME J 6(7), 1415-1426.

ACCEPTED MANUSCRIPT

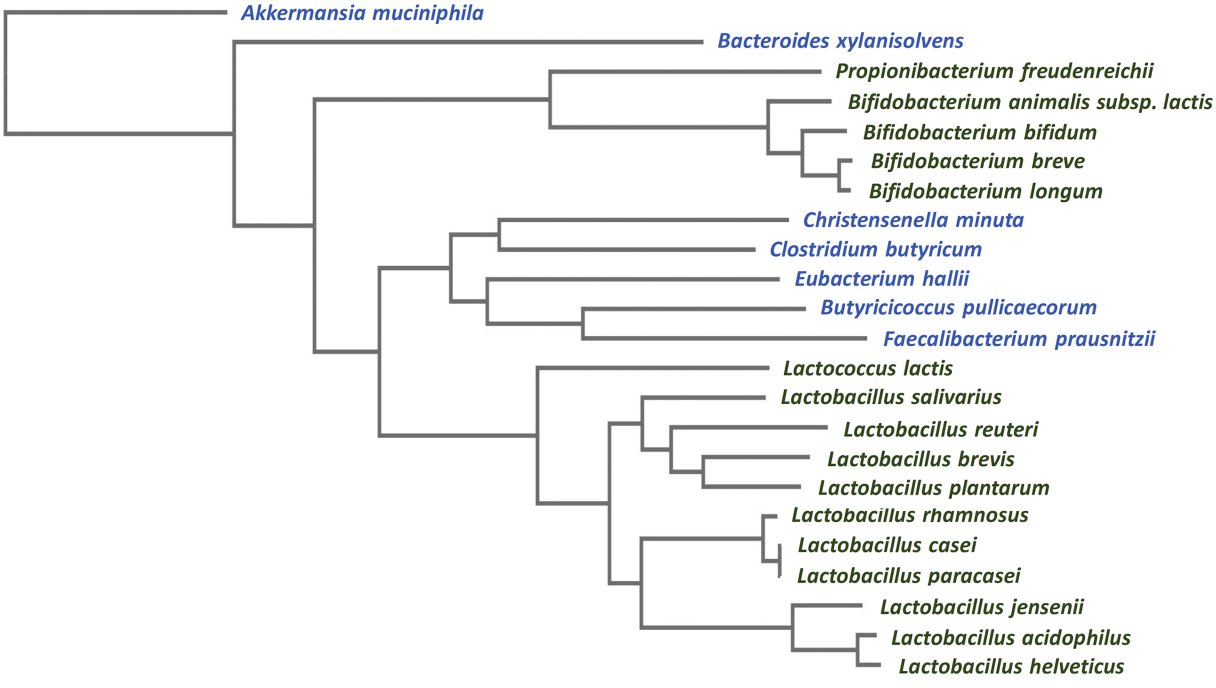
Akkermansia muciniphila
Bacteroides xylanisolvens
Propionibacterium freudenreichii
Bifidobacterium animalis subsp. lactis
Bifidobacterium bifidum
Bifidobacterium breve
Bifidobacterium longum
Christensenella minuta
Clostridium butyricum
Eubacterium hallii
Butyricicoccus pullicaecorum
Faecalibacterium prausnitzii
Lactococcus lactis
Lactobacillus salivarius
Lactobacillus reuteri
Lactobacillus brevis
Lactobacillus plantarum
Lactobacillus rhamnosus
Lactobacillus casei
Lactobacillus paracasei
Lactobacillus jensenii
Lactobacillus acidophilus
Lactobacillus helveticus

Figure 1

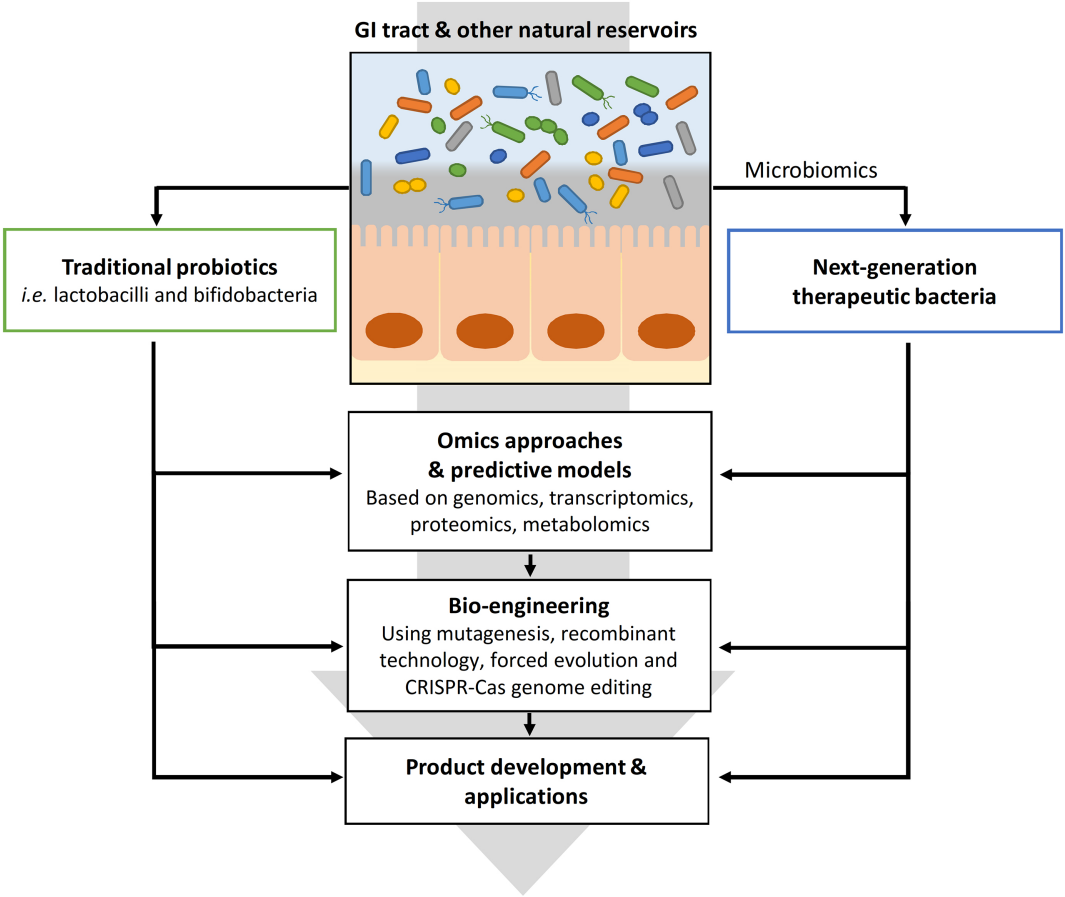
GI tract & other natural reservoirs
Microbiomics
Traditional probiotics
i.e. lactobacilli and bifidobacteria
Next-generation therapeutic bacteria
Omics approaches & predictive models
Based on genomics, transcriptomics, proteomics, metabolomics
Bio-engineering
Using mutagenesis, recombinant technology, forced evolution and CRISPR-Cas genome editing
Product development & applications

Figure 2

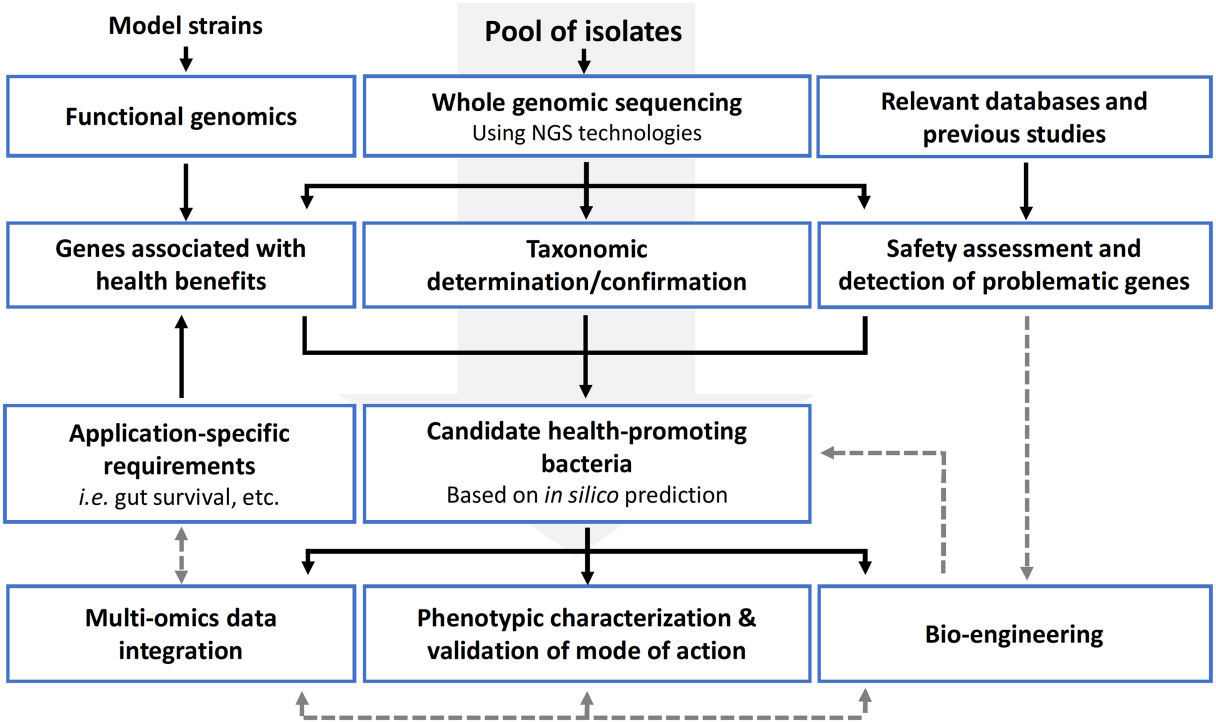
Model strains
Pool of isolates
Functional genomics
Whole genomic sequencing
Using NGS technologies
Relevant databases and previous studies
Genes associated with health benefits
Taxonomic determination/confirmation
Safety assessment and detection of problematic genes
Application-specific requirements
i.e. gut survival, etc.
Candidate health-promoting bacteria
Based on in silico prediction
Multi-omics data integration
Phenotypic characterization & validation of mode of action
Bio-engineering

Figure 3

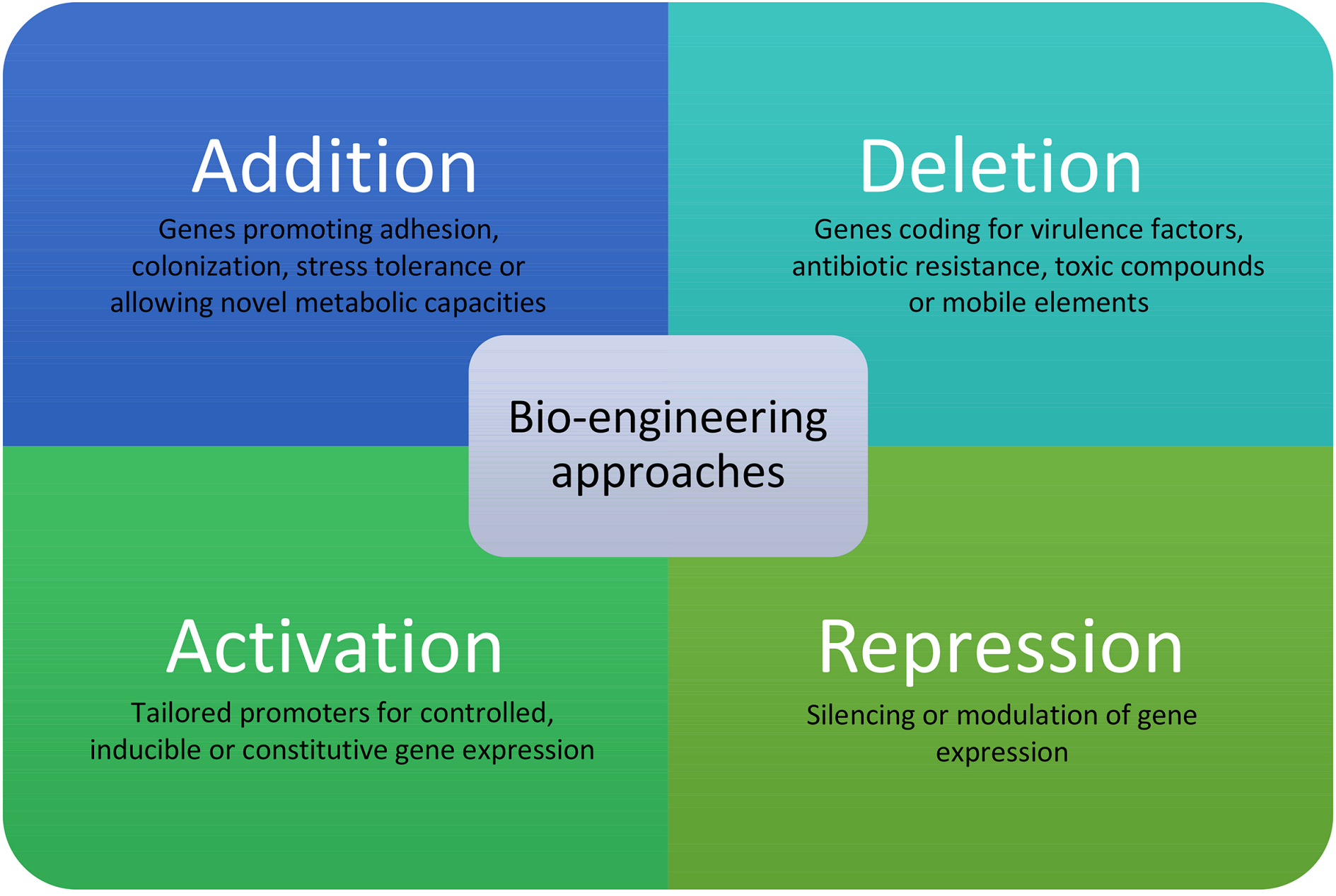
Addition
Genes promoting adhesion, colonization, stress tolerance or allowing novel metabolic capacities
Deletion
Genes coding for virulence factors, antibiotic resistance, toxic compounds or mobile elements
Bio-engineering approaches
Activation
Tailored promoters for controlled, inducible or constitutive gene expression
Repression
Silencing or modulation of gene expression

Figure 4