

Probiotics: common genera, mechanisms, current phase and the future

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Abstract. Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. This review provides an introduction to the current probiotics field, including their isolation and engineering, some common genera, their actions, and their applications in industry and public. We discuss the source of isolation and techniques in engineering probiotics, their role in modulating gut health, evidences of their application in treating gastrointestinal disorders, and slightly covers their application outside of human. We also discussed their actions on gut microbiota, epithelial environment, and human gut-brain axis. Finally, we address the limitation of current probiotic products and their application, and brings up the future research directions in this field. The purpose of this paper is to provide an introductory review on the current probiotic field.

Keywords: Probiotics, gut microbiota, immune modulation, shorty-chain fatty acids.

1. Introduction

The human gastrointestinal tract is a complex ecosystem that harbors over 100 trillion of more than 300 species of bacterial species [1], namely the gut microbiota. Gut microbiota is composed of microorganisms from all three kingdoms with 30–40 species consisting 99% of the community [2]. Bacteria alone contributed to at least 90% of all microorganisms in human GI tract and is generally comes from about ten phyla of microorganisms, with Firmicutes and Bacteroidetes being the most represented phyla [3], making it the most complicated biosystem in human body.

Gut microbiota is considered as the second human genome pool which extend the coded information more than 150 times compared with human genome itself [4]. Some of these genes are crucial to human health. For example, some strains in *Lactobacillus plantarum* are able to produce vitamin Bs, an essential vitamin family that cannot be produce by human body [5]. Vitamin Bs deficiency is responsible to many diseases such as seizures, hair loss, and neural tube defect [6, 7]. Microorganisms such as *Lactobacillus plantarum* that improve health conditions in human GI tract are not rare. Together, they are called “Probiotics”.

Probiotics are defined as live microorganisms that bring health benefits to the host when administered in adequate amount [8]. For example, in addition to *Lactobacillus plantarum*, *Lactobacillus acidophilus* strains are known to control cholesterol level [9], ease lactose mediated diarrhea [10], and regulate inflammation response [11]; *Saccharomyces boulardii* strains could degrade pathogenic toxins [12], increase immune response [13], and inhibit pathogenic growth and translocation [14, 15].

This review will provide an introduction on the probiotic topics and discuss two types of probiotics, namely the natural probiotics and engineered probiotics, common probiotics genera and their bio-significance and applications, actions and mechanisms of probiotic functions, and the current struggle and futures of probiotic field.

2. Natural probiotics

Natural probiotics are probiotics that are not modified through gene editing. They are naturally found in daily food in the human dietary system, especially fermented foods. Examples are: yogurt,

cheese, beverages, fermented tofu, etc. [16, 17] Natural probiotics have been selected and co-evolved with humans for centuries and therefore are safe for human consumption.

Natural probiotics are commonly isolated from fermented foods, animal breast milk, and human feces samples in laboratory settings, which involves a series of steps of sample collection, microorganism isolation, strain identification by 16s RNA sequencing [18], and strain categorization. Common bacterial genera that are considered to have probiotic potentials include *Lactobacillus* (found in dairy products such as cheese and yogurt, and all kinds of pickles) [19], *Bifidobacterium* (inhabitant of animal gastrointestinal tract and vagina) [20], *Saccharomyces* (used in fermented beverages such as wine and beer, and bread) [21], *Bacillus* (used in various of treatments such as in irritable bowel syndrome and bladder cancer) [22]. While some gram-negative strains like *E. coli* Nissle 1917 are approved to have probiotic functions [23], excluding fungi, most of the probiotics that are recognized in current industry are gram-positive [24].

Once a strain is identified and classified with probiotic potential, probiotic utilities are then assessed. To be considered as a candidate for clinical trial, one strain must exhibit certain probiotic criteria. Conventional assessment includes tests on pathogenicity and toxicity, pH, heat, and bile salt tolerance, adhesion to human epithelial cells, inhibition of pathogenic adhesion to human epithelial cells, and in vitro survivability [25]. To assess the pathogenicity and toxicity of a strain, identifying virulence factors in the corresponding strain through genome sequencing and RNA-seq is the most common approach [26]. Virulence factors are partially unique to each genus: having both shared virulence factors among different genera such as adhesins and toxins, and unique virulence factors that are only present in a few corresponding genera. Examples are Staphylococcal Protein A in *Staphylococcus* (binds to the Fc receptor of the host immunoglobulins and interferes with their immune response) [27] and Streptokinase produced by *Streptococcus* (converts plasminogen to plasmin, which degrades tissue fibrin clots and promotes invasion) [28]. Other probiotic functions such as antimicrobial activity are also commonly tested along the series of assessments.

Although being a “natural” strain often indicates a relatively easy integration, natural probiotics have several limitations. Environments in human gastrointestinal tracts are often different from other mammals or fermented foods and even from person to person, unmodified strains often exhibit a low survival rate in the human gastrointestinal system [29]. Reduced probiotic effects or compromised probiotic functions are also common phenomena in clinical trials and applications [30]. While it is true that most natural probiotic products are considered safe, contamination by pathogenic strains is also a presenting issue in the industry. One study from 2019 found more than 42% of probiotic supplements contained unwanted microorganisms [31]. Despite these limitations, natural probiotics remain a popular choice in probiotic development and are considered a relatively mature field biology industry.

3. Engineered probiotics

Engineered or artificial probiotics are probiotics that are modified through gene editing. In responding to the low survival rate of natural probiotics in human guts and compromised functions, and to selectively introduce health-beneficial functions that are not commonly present in natural strains, the industry expanded to gene editing and engineering. Followed by the development of the recombinant DNA technique in the 1970s by Stanley Cohen et al. [32], the idea of genetically modifying bacterial strains to obtain beneficial functions emerged in the late 20th century. In 2000, the pioneering research led by Steidler was able to engineer *Lactococcus lactis* to secrete interleukin-10, an anti-inflammatory cytokine, to treat colitis in mice [33], and is one of the earliest examples of engineered probiotics.

The techniques of engineering bacterium evolved along with the development of gene editing. Homologous recombination was one of the first gene editing methods widely applied [34]. In the 2000s and early 2010s, engineered zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were widely applied [35], but the unspecificity and laborious

procedures limited their application [36]. Scientists moved on to a much more powerful gene editing technology, CRISPR, in the mid-2010s, and are now able to perform precise gene excision and insertion by designing crRNA and tracrRNA and converting them to sgRNA that guide the Cas nucleases to specific sites. CRISPR-Cas9 technology is still the newest and most powerful tool in gene editing.

Current engineered probiotics development uses a wide range of template bacterial genera including *Lactiplantibacillus*, *Lactiacaseibacillus*, *Lactococcus*, *Lactobacillus*, *Limosilactobacillus*, *Bifidobacterium*, *Escherichia*, etc. [37, 38] and achieves probiotic therapeutic functions by expressing exogenous proteins, cytokines, peptides, and vaccine macroparticles while eliminating pathogenic and toxic features [39, 42].

Lactobacillus and *Lactococcus* are two common probiotic templates derived from fermented foods and dairy products. They both have strains that are naturally found with probiotic functions and are widely applied in both the food industry and therapeutic uses [40, 41]. The “natural” aspect makes them relatively safe when serve as a template for gene-edited probiotics and are heavily investigated on their potential utilities. For example, multiple *Lactobacillus* strains *L. acidophilus* ATCC 4356 and *L. d. subsp. lactis* D17 are engineered as vaccines to carry both viral and bacterial protein particles [42, 43], and multiple *Lactococcus* spp. are engineered to express superoxide dismutase to reduce oxidative damages, to express cathelicidin for anti-inflammatory effects, or to express Hsp65 to prevent atherosclerosis [37, 45, 46, 47]. While it is true that *Escherichia coli* is the most well-known pathogenic species, the strain *E. coli* Nissle 1917 is one of the most common probiotic templates used due to its well-understood genome and proven safety [23, 48]. It has been engineered and proposed for a role in developing the treatment of cancer [49], inflammatory bowel disease [50], hyperammonemia [51], and many other diseases. Together, these provide a view of the potential and foreground of the engineered probiotics

A broad spectrum of promising functions and enhanced expression are indeed some advantages of engineered probiotics, but they still face several limitations. Examples are reduced genome stability caused by the presence of multiple gene editing sites [52], incomplete deletion of large sequences that encode unwanted features [53], and outcompete the native strains and largely altering gut microbiota composition [54]. Engineering probiotics is still an emerging field that requires further studies and complete regulations.

4. Common probiotic genera

4.1. Bifidobacterium

In modern industry and research, one of the most examined probiotics genera is the *Bifidobacterium*, an anaerobic bacterial genus commonly found in mammal’s gastrointestinal tract [55]. *Bifidobacterium* is the most common microbiota in infant stage and is one of the earliest microbiotas colonize in infant. It has been discovered that breast-fed infants express earlier colonization of *Bifidobacterium* than formula-fed [56]. *Bifidobacterium* species such as *B. longum* and *B. breve* are known to be able to hydrolyze milk oligosaccharides, which are not digested by human’s own gastrointestinal tract [57]. Applications of probiotic *Bifidobacteria* are heavily investigated in a wide range of field. Bifidobacterial microbe’s colonization is shown to outcompete pathogens and provide anti-infectious activities in human gastrointestinal tract [58], respiratory tract [59], and animal’s gut [60]. They are also known to stimulate both innate and acquired immune responses to viral infection [61]. *Bifidobacterium* has been trialed as an anticancer strategy for years, for example, *B. longum* BCRC 910051 was shown to exhibits inhibition of growth colon tumor [62]. Bifidobacterial metabolism produces neuroactive metabolites such as hypoxanthine and tryptophan [63], expanding the potential of *Bifidobacterium* to the field of psychological health. *B. breve* CCFM1025 [64] and *B. longum* 1714 [65] are known to reduce stress and reverse stress-induced depression on mice.

4.2. *Lactobacillus*

Lactobacillus is a genus of aerotolerant anaerobe bacteria. Colonies of *Lactobacillus* are found in a number of areas in human, including gastrointestinal track and female genital system, and protect them from external pathogens [66]. *Lactobacillus* is also known to the metabolism that produce lactic acid from carbohydrates, and it is the largest LAB genus and shows extremely wide range of genotype and phenotype diversity [67]. This nature makes them one of the most common probiotics found in ferment food such as yogurt. *Lactobacillus* is also heavily investigated in medical and biochemical fields. By competing intestinal attachment, producing carcinogen inactivators, and provoking immune responses, probiotics are believed to decrease the risk of tumorigenesis [68]. *L. casei* LC9018 was found to be potentially reducing colon cancer risk by altering gut microbiome [69]. Species such as *L. reuteri* CRL 1098 are shown to have ability to enhance the response of ochratoxin A, a food mycotoxin produced by *Aspergillus* and *Penicillium* [70]. *L. kefir* CIDCA 8348 and JCM 5818 showed protective activities from cadmium cations [71]. As the dominating microbes in female vaginal tract, *Lactobacilli* is critically important in maintaining microbiota balance. Deficiency of *Lactobacilli* may lead to proliferation of pathogens and bacterial vaginosis, which is the most common vaginal diseases and is the potential precursor of urine track infection and unhealthy newborns [72]. *Helicobacter pylori* is a common human gastric pathogen and is the main cause of major chronic gastric diseases and cancer [73]. Investigation of *Lactobacilli* as treatment of *H. pylori* has been undergoing for decades, and multiple strains has been suspected to neutralize *H. pylori* [74, 75]. Studies also showed that *L. plantarum* Lp299 [76], CUL66, *L. acidophilus* CUL60 and CUL21 [77] can decrease the risk of respiratory infection. It is also suggested that *L. casei* Shirota can improve aerobic capacity in sport players [78].

4.3. *Enterococcus*

Enterococcus is another large genus of lactic acid bacteria that holds both extensive amount of probiotic and pathogenic species to human. They are found in both mammal's and insect's gut, and in natural biome such as water and soil [79, 80]. Pathogenic behaviors of *Enterococcus* have been well studied and recorded for decades. They have been shown to responsible for multiple hospital-related infection, including urinary tract infection and endocarditis [81]. It is estimated that it caused 14% hospital-acquired infection in the United States between 2011 and 2014 [82]. On the other hand, its probiotic behaviors are also a frontier field in current industry. The bacteriocins produced by multiple *Enterococci* strains are well characterized, and their antimicrobial ability is considered a strategy to reduce the number of competing pathogenic microbes in gastrointestinal track [83]. These bacteriocins are considered a potential replacement of current antibiotics to treat drug resistant pathogens [84]. Bacteriocin from *E. faecalis* SL5 has been applied in the treatment of acne and shows little toxicity on host cells under low concentration [85]. Several *Enterococcus* strains such as *E. faecium* SF68 have been proved effectiveness and safety and are included in several treatment for human irritable bowel symptoms and diarrhea [86]. *Enterococci* are also crucial to ferment food industry. *E. faecium*, *E. faecalis*, and *E. durans* are commonly found in fermented milk products [87], and their thermostable enterocins and bacteriocins are important for preserving milk and cheese against foodborne pathogens such as *S. aureus* [88]. Aside from dairy industry, bacteriocin-producing *Enterococcus* strains are also applied in ferment meat products such as sausages and dried seafood [89]. But their ability to compete with pre-existed pathogenic bacteria like *E. coli* and *L. monocytogenes* [90].

4.4. *Bacillus*

Bacillus is a genus of bacteria that express both aerobic and anaerobic species [91]. They can survive a wide range of harsh condition due to their ability to form endospores [92], making them adaptive in many environments, including aqua ecosystem, soil, animal's gut, and even in well-prepared food. Common *Bacillus* probiotics include *B. cereus* [93], *B. clausii* [94], *B. coagulans* [95], and *B. licheniformis* [96]. *B. cereus* is one of the common food pathogen, but it is also used as

probiotics in human and livestock. The safety concerns of *B. cereus* remain a debatable topic in industry, and their toxin production pathogenic mechanisms are still under investigation [97]. *B. clausii* are known to modulate human immune response and are effective against urinary tract infection [98] and inflammation of *H. pylori* therapy [99]. Due to its natural occurrence in waters and soil, probiotic *Bacillus* are also applied in agriculture. *B. licheniformis* was proposed to mediate secretion of bacitracin [100] and synthesis bacterial cytokinins [101]. It is also able to increase corn's drought tolerance [102] and cucumber's salinity tolerance [103].

5. Actions of probiotics

5.1. Maintain microbial Balance by antimicrobial compound and competitive exclusion

Microbial balance by definition is the balance of the microorganism colonies that occupy host tissue [104], with both the probiotic and pathogenic microorganisms involved. Studies have demonstrated multiple human diseases and disorders related to the changes in microbial balances such as allergic and asthma [105], autoimmune disorders [106], inflammatory bowel diseases and irritable bowel diseases [108], and obesity and diabetes [107]. Dysbiosis, the reduction in microbiota diversity and the loss of beneficial bacteria, is the main cause of them [109]. Two major mechanisms by which probiotics participate in gut microbial balances are competitive exclusion and antimicrobial compound secretion [110, 111].

Competitive exclusion refers to the action of a bacteria species to exclude another species by competing for the occupation chance of attachment receptors in gut epithelial cells [112]. Numerous pieces of evidence have shown that probiotic strains are capable of inhibiting the adhesion of toxins and pathogens to the epithelial receptors in human guts in a strain-specific manner. *Limosilactobacillus reuteri* ATCC PTA 6475 and ATCC 53608 were proven to inhibit Enteropathogenic *Escherichia coli* binding to HT-29 cells [113]. *Bifidobacterium lactis* HN019 was able to inhibit *Salmonella enterica subsp. enterica* ATCC 14028 attachments to INT407 cells [114]. *Lactobacillus crispatus* strains K313 and K243 exhibited an inhibition role on adherence of *Salmonella braenderup* H9812 to HT-29 [115]. The detailed mechanism of receptor-related competitive exclusion is strain specific and is largely unknown, but is generally believed to involve bioactive molecules such as peptidoglycan, lipoteichoic acid [116], fibronectins [117], and surface-bound proteins [118]. One study in 2016 demonstrated a similar structure between *Enterococcus faecium* E1165 and *Lactobacillus rhamnosus* GG mucus-binding pili at the genomic level and enables competition between two strains in gastrointestinal mucus-binding [119].

Antimicrobial peptides (AMPs) are a group of small peptides that play a crucial role in the innate immune response of organisms in all six kingdoms [120]. It is an emerging tool in treating microorganism-related diseases in responding to the development of antibiotic-resistant strains in chronic administration of canonical antibiotic treatment [121]. AMPs in particular strains such as *Lactobacillus plantarum* MTCC 1407 and *Lactobacillus acidophilus* MTCC 10307 are found to be effective even in treating drug resistance enteroaggregative *Escherichia coli* [126]. AMPs are naturally synthesized in many probiotic genera including *lactobacilli* [124], *lactococcus* [125], *bifidobacteria* [123], and many other less popular genera such as *Pediococcus* [125], and are both applied in species-specific treatments and as broad-spectrum bacteriocin contributor. AMP expression can also be engineered into bacterial strains that do not possess them naturally by plasmids, giving a variety of possible functions. *Escherichia coli* Nissle [127], *Lactococcus lactis* [122], and *Lactocasei bacillus casei* [128], are some of the common templates.

5.2. Gut Barrier functions and mucus maintenance

The gut barrier is a multilayer system consisting of epithelial cells and mucus that selectively allows permeation of nutrients and essential biological functions while excluding pathogens from entering the body [129] in a similar to the manner in which stratum corneum protects human bodies from alien microbial invasion [130]. AMPs, secretory immunoglobulin A, and mucins are the major

components of gut mucus [131, 132]. The most abundant mucin protein in small and large intestines is mucin 2, and its knockout has been shown to cause colitis and neuroinflammation in mice [133, 134]. Other mucin types also serve in differentiated roles such as epithelial cell protection and gel formation [135].

The mucus layer in the human gut barrier is a sensitive system that is being degraded and replenished constantly. The gut microbiome serves a major role in mucus maintenance. Studies have shown that pathogenic species such as *Clostridium difficile* and *Helicobacter pylori* are responsible for inhibiting mucus secretion by blocking the synthesis and exocytosis of mucin [136, 137]. Other factors such as diet are also the reasons for mucus compromising. One study showed that a low-fiber diet is positively related to the growth of *Bacteroides caccae* and *Akkermansia muciniphila*, both of which are mucin-degrading species [138]. Numerous probiotic strains have been investigated for their mucus maintenance functions. *Lactobacillus plantarum* 299v induced an increased expression in mucin 2 and 3 on HT-29 cells with a decreased adherence of foreign *Escherichia coli* [139]. *Lactobacillus casei* GG was able to stimulate the expression of mucin 2 and reduce *Escherichia coli* translocation [140]. *Lactobacillus reuteri* R2LC and *Lactobacillus reuteri* 4659 exhibited a thickening of mucus and induced expression of tight junction proteins occludin and ZO-1 [141]. *Bacteroides fragilis* ZY-312 motivated the STAT3 signaling pathway and mediated colonic mucosa regeneration [142].

5.3. Immune responses regulation

The intestine microbiota has co-evolved with mammals for millennia along with the evolution of diets and the pathogens that came with it, and the intestine itself is considered the largest and the most important immune organ in mammals [143], including humans. The immune system in the human intestine is a complex system where innate responses and adaptive responses cooperate to protect the host from pathogens and toxins. Nonspecific innate immunity is related to the “barriers” in the intestine such as the chemically active mucus and the physical epithelial cell and also includes innate immune cells such as macrophages and natural killer cells [144]. Adaptive immunity is a much more specific mechanism against pathogens and typically involves the cooperation between multiple immune factors such as lymphocytes B cells and T cells, antigen-presenting cells, dendritic cells, as well as many cytokines [145, 146, 147].

Immunomodulatory function is one criterion of probiotics [148] and is generally accomplished by interacting with the immune factors in both innate and adaptive mechanisms. Corresponding machineries have been well studied and documented with strain specificity. *Lacticaseibacillus paracasei* NCC2461 could promote anti-inflammatory effect by inhibiting the development of CD4+ T cells with inhibited Th1 and Th2 cytokines secretion while maintaining IL-10 and TGF- β secretion [149]. *Lactiplantibacillus plantarum* OLL2712 enhanced IL-10 production from intestinal dendritic cells [150]. *Lactobacillus helveticus* LH2171 inhibited the activation of nuclear factor-kappa B and mitogen-activated protein kinases and induced A20 expression through TLR2 signaling and exhibited an anti-inflammatory effect on encephalomyelitis [151]. *Escherichia coli* Nissle 1917 decreased intestinal inflammation by inhibiting the expression level of IL-2, tumor necrosis factor α , and gamma interferon while increasing IL-10 production in peripheral blood T cells in a TLR-2 dependent manner without reducing T cell immunity below functioning level [152]. Edited lactic acid expressing *Saccharomyces cerevisiae* BY4741 suppressed polarization and NLRP3 inflammasome activation in M1 macrophages by decreasing the mRNA expressions of the iNOS and IL-6 and by decreasing IL-1 β and caspase-1 respectively [153]. *Lactobacillus reuteri* supplement [154] and *Lactobacillus rhamnosus* GG ATCC 53013 were proven to be effective in treating retrovirus-related gastroenteritis and diarrhea. Probiotic strains from various genera are also shown to be effective in parasitic treatments by accelerating immune responses to multiple common parasites [155].

5.4. Short-chain fatty acid

Short-chain fatty acids (SCFAs) are defined as fatty acids with less than six carbons and are the main metabolites from anaerobic microbiota in the human gut [156, 157]. Most of the SCFAs found in guts are acetic acids (C2), propionic acids (C3), and butyric acids (C4) as they can be synthesized from pyruvate, succinate, and acetyl-CoA respectively, which all are essential intermediates or end products for cell metabolism in both eukaryotes and prokaryotes [158, 159]. Lactate and SCFAs-producing microorganisms are commonly found in the human gastrointestinal tract. Some famous species are *Faecalibacterium prausnitzii*, the major butyrate producer [164], *Bifidobacterium longum*, an acetate producer that also produces butyrate by consuming acetate [165], etc. Functions of short-chain fatty acids have been well studied and described for decades, for example, buffering gut pH [160], regulating gut absorption of metal ions [161], modulating brain-gut axis [166], and functions mentioned above such as immunomodulation [162] and anti-inflammation [163]. Because of the broad spectrum of health benefits brought by short-chain fatty acids, probiotic strains that produce them constitute a significant portion of all probiotics.

Most pathogens prefer neutral pH and the acidic environment found in the human gastrointestinal tract and vaginal tract is not friendly to them. The functions and benefits of low pH brought by SCFAs have been well described by previous studies. SCFAs are also important “foods” of epithelial cells and immune cells and are linked to their proliferation regulation [167]. Butyrate in particular is known to suppress colon tumorigenesis [168]. Antimicrobial and antibiotic treatment efficiencies were shown to be dependent on gut pH as well [169, 170]. The mechanism was proposed to be related to a strengthened TCA cycle and the promotion of pathogenic metabolite and its interaction with drugs under a more acidic environment [171]. Many probiotic strains are known to reduce the pH of the host gastrointestinal tract and promote beneficial outcomes. For example, *Lactobacillus acidophilus* KFR1342 can inhibit the growth of *Escherichia coli* and reduce the production of these enzymes, but can also inhibit carcinogenic enzyme activity [172]; multiple probiotic *Bifidobacteria* species were able to produce enough amount of acetic acid to lower the pH of intestine and inhibit Shiga toxin production in *Escherichia coli* O157:H7 [173].

Metal ions are important cofactors in many biological pathways and their deficiency could lead to multiple common diseases. Some examples are anemia and fatigue (caused by iron deficiency), osteoporosis and delirium (caused by calcium deficiency), and arrhythmia (caused by magnesium deficiency). Short-chain fatty acid's role in metal ion absorption has been well described. The acidification of the gut lumen is thought to be helpful in metal ion formation and absorption such as Mg^{2+} [174]. SCFAs also are shown to be effective in promoting transient receptor potential cation channel expression. Propionate and butyrate could largely elevate the mRNA level of TRPV6, which is responsible for calcium absorption [175]. Although not as strong as most amino acids or ascorbic acid, SCFAs are capable of lowering the pH and reducing ferric iron to its ferrous form, which is more bioavailable for absorption [176]. The short-chain fatty acid-secreting probiotic that promotes the absorption of essential metals is a hot topic in the field. *Lactobacillus plantarum* 299v was shown to be capable of increasing iron bioavailability in women [177]; *Lactobacillus plantarum* C4 increased calcium bioavailability in goats' milk [178]. *Lactobacillus casei* Shirota significantly increased iron metabolism of intestinal microflora [179]; *Lactobacillus casei* 393 increased calcium level and bone strength in rat femur [180].

The gut-brain axis is the biochemical signaling channel connecting the brain and gastrointestinal nervous system [181]. The idea of the communication between the gut nervous system and the brain rose no later than the 1980s as people found peptides and hormones in both the central and gastrointestinal nervous systems, and the term “gut-brain axis” has been mentioned since early 1980 [182]. Some of the pioneering findings proved the existence of such communication. In 1998, scientists found an elevated anxiety-like behavior triggered by the treatment of pathogenic *Campylobacter jejuni* in mice [183], providing direct evidence of gut-mediated central nervous system regulation. To our knowledge today, the most important microbiota metabolites in the gut-brain axis are short-chain fatty acids. SCFAs, especially acetates, are important energy sources of

nerve cells [184]. They also regulate gut hormone release [185]. Most importantly, SCFAs are known to be capable of crossing the brain-blood barrier and regulating brain functions [185]. Because of the capability of SCFAs to regulate nerve and mental health, treatment of mental disorders such as anxiety and depression with SCFAs-secreting probiotics are a hot topic in the field, and many strains exhibited promising effects in both laboratory settings and clinical trials. A human trial with a combined probiotic treatment containing multiple *Bifidobacterium*, *Lactobacillus*, and *Lactococcus* strains was shown to reduce aggressive and rumination mood (n=20) [186]. Postpartum depression and anxiety were significantly decreased by *Lactobacillus rhamnosus* HN001 in a double-blinded trial (n=423) [188]. A probiotic tablet developed by Xinyi Pharmaceutical Factory containing mainly *Bifidobacterium longum*, *Lactobacillus bulgaricus*, and *Enterococcus faecalis* was proven to be effective in helping patients recovering from severe traumatic brain injury (n=76) [187].

6. Current struggle and future

In recent years, the development and expansion of the probiotic field have been widely widely-recognized in both academia and society. It also raised several questions and struggled the field. Lack of regulation and poor quality control are the most important impactors to the current probiotic industry [189]. Since the early 2000s, multiple reports and clinical trials have stated the lack of evidence of the proposed effects in corresponding probiotic products [190], and some of time even reported undesirable adverse effects such as increasing mortality [191]. Most cell cultures and in vitro mechanical trials are often single-property focused and lack the complexity that is normally found in life, and short-term in vivo experiments on murine cannot reflect the chronic consequences of the proposed treatment. The most tragical example, although not probiotic related, is thalidomide, which is designed as a sedative for pregnant women and passed short-term trials but was later found toxic under chronic treatment [138]. Thermo-instability in storing and consumption is another challenge for current commercial probiotic supplements [194], and the compromising effect or deactivation of functional bioproducts under heat or freezing is widely recorded [136]. Strain specificity of effects and nonuniversal gut microflora are also important challenges of safe and effective probiotic application [192]. Different gut internal environments from patient to patient, such as pH values, might affect the activity of bile salt hydrolases produced by probiotics, which further impair lipid metabolism and cause gain of weight [193]. Other physical conditions immunocompromising, mental stress, and age might switch probiotic strains to lethal pathogens [194]. Therefore, developing better quality assurance technologies, culturing thermo-tolerant strains, and personalizing probiotic treatment are likely three of the most essential directions. A comprehensive testing cell culture model that simulates the gut environment and reflects the true microbial response, even derived directly from patients, should be developed and applied in future probiotic research.

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