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Review

Trends, Functionalities, and Prospects of Probiotics

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The importance of beneficial microorganisms, particularly probiotics, that coexist in the human body, is being increasingly recognized. Probiotics are representative health functional foods that provide health benefits to humans through the production of various metabolites, including short-chain fatty acids. However, the health benefits are strain-specific, and the use of each probiotic strain should follow guidelines that assure its safety. Accurate identification of the strain should be managed through genetic and phenotypic analyses of the strain. Besides, the functionality of probiotics should be disclosed in vitro and in vivo so that they can be used as legal functional ingredients (i.e., individual standards). In this review, we deal with the guidelines, including the technical factors related to probiotic strains. The common health effects of probiotic strains include proliferation of beneficial bacteria, control of harmful bacteria, and facilitation of bowel activities. Probiotics with various functionalities (e.g., body fat and cholesterol reduction, vaginal health, and improvement of skin's immune system) have been investigated as "individual standards of raw materials for health functional foods" provided by MFDS. In the future, various biotechnologies including synthetic biology can be applied to produce customized probiotics to improve human health.

Keywords: Probiotics, Health benefits, microbiome, commercialization

Introduction

Recently, markets for health functional foods have expanded with an increase in consumer' interests, particularly after the COVID-19 pandemic, to improve immunity [1]. According to a survey conducted by the Natural Marketing Institute in May 2020, the consumption of probiotics has significantly increased since the 2019 pandemic, with a 66% increase in consumers in the United States and a 108% increase in China compared to the previous year [2]. In addition, the 2021–2030 Probiotics Market Analysis Report released by Precedence Research expects that the global probiotics sales market will reach approximately $63.1 billion in 2021, and with 8.7% annual growth, reaching approximately $133.9 billion by 2030 [2]. In Korea, the probiotics-' market provided by the Ministry of Food and Drug Safety (MFDS) is continuously growing from 217.4 billion won in 2017 to 525.6 billion won in 2020 based on annual sales [3]. Probiotics have become one of the most popular food supplements to many consumers, and their various functionalities include skin care, immune enhancers, and anti-diabetes [4]. Thus, in this review, the current trends in the probiotics industry in terms of functionalities and standard criteria for manufacturing are broadly discussed.

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History of Microbial Research

The microorganisms was firstly discovered in the late 1600s by Antonie van Leeuwenhoek, a Dutch scientist, who observed "microanimals (animalcules)" through a single-lens microscope [5]. His observations revealed the presence of various microorganisms in human feces, teeth, and rainwater, but did not provide any insights in biological associations with microorganisms [6, 7]. Robert Hooke FRS, a British scientist, firstly used the term "Cell" in "Micrographia" [8]. Marcus von Plenciz, a Slovenian physician, suggested the relationship between microorganisms (i.e., bad air and miasmas) and diseases by germ or contagion theory published in Opera medico-physica 1762 [9]. Louis Pasteur, the father of microorganisms disproved spontaneous generation through theory of biogenesis by performing swan neck flask experiment in 1861 [10]. In 1876, Robert Koch discovered that anthrax was caused by rod-shaped bacteria (now known as *Bacillus anthracis*). Since then, fungi, viruses, and protozoans have been revealed as causes of diseases, and microorganisms have been widely recognized as harmful to humans based on germ theory [11]. However, since 1857, when Louis Pasteur discovered lactic acid bacteria in wine, causing alcoholic ketoacidosis, the benefits of microorganisms began to be discussed for the first time through his publication, "Microorganism vs. disorder 1857 (lactic acid production)" [12]. Later, Elie Metchnikoff suggested that decay in the large intestine was the cause of human aging at "The prolongation of Life" in 1908 and that Bulgarian yoghurt could delay aging caused by harmful bacteria with the aid of lactic acid bacteria [13]. In other words, a new direction of microorganism studies has been proposed to help human health as beneficial bacteria.

Prerequisites for Commercial Probiotic Production

Definitions and nomenclature

The etymology of probiotics is derived from the Latin "pro", meaning "for" and the Greek "bios" meaning "life". In 1953, Werner Kollath, a German bacteriologist, defined it as the opposite of antibiotics, which means killing bacteria. He described that "Restoring the health of patients suffering from malnutrition caused by excessive consumption of highly refined food requires organic and inorganic supplements." [15, 16]. With the large growth of their market, the World Health Organization (WHO) defined the term "probiotics" as 'live microorganisms that are intended to have health benefits when consumed or applied to human body' by the Food and Agricultural Organization of the United Nations (FAO) and the International Scientific Association for probiotics and prebiotics (ISAPP) in 2002 [17]. Accordingly, the MFDS in the Republic of Korea defined probiotics as "living bacteria that have health benefits."

Lactobacillus, Bifidobacterium, Streptococcus, Enterococcus, Bacillus, Escherichia, and *Saccharomyces* are common probiotics [18]. Among them, *Lactobacillus* and *Bifidobacterium* are the two major genera. For example, *L. acidophilus, L. casei, L. paracasei, L. plantarum, L. rhamnosus, L. bulgaricus, L. gasseri*, and *L. reuteri* for *Lactobacillus* species are considered the most important strains with their benefits (the functionalities of these species will be discussed below) [19]. Recently, *Lactobacillus* genus were reclassified into 25 genera using DNA-based analytical tools with physiological and metabolic properties, including *Acetilactobacillus, Amylolactobacillus, Agrilactobacillus, Apilactobacillus, Bombilactobacillus, Companilactobacillus, Dellaglioa, Fructilactobacillus, Furfurilactobacillus, Holzapfelia, Lacticaseibacillus, Lactiplantibacillus, Lactobacillus delbrueckii* group, *Lapidilactobacillus, Latilactobacillus, Lentilactobacillus, Levilactobacillus, Ligilactobacillus, Limosilactobacillus, Liquorilactobacillus, Loigolactobacillus, Paucilactobacillus, Paralactobacillus, Schleiferilactobacillus,* and *Secundilactobacillus* (Table 1).

Prerequisites for probiotic production

The MFDS in the Republic of Korea recommend several criteria, such as acid tolerance, bile acid tolerance, proliferation, adhesion to the intestine, effective functionality in the intestine, and non-pathogenic and non-toxic properties [14]. In other words, safety, functionality, and stability are prerequisites for probiotic strains (Table 2). First, the safety of the strain is based on its origin, absence of pathogenicity, and antibiotic resistance. Second, the functionalities of the strain are related to its viability and immune control effect in the gastrointestinal tract. Stability refers to the survivability of the

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Table 1. Reclassified taxonomic genera of representative probiotic strains.

Genus	Species
Lactobacillus	*Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*
Lacticaseibacillus	*Lacticaseibacillus casei*, *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*
Limosilactobacillus	*Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*
Lactiplantibacillus	*Lactiplantibacillus plantarum*
Ligilactobacillus	*Ligilactobacillus salivarius*
Lactococcus	*Lactococcus lactis*
Enterococcus	*Enterococcus faecium*, *Enterococcus faecalis*
Streptococcus	*Streptococcus thermophilus*
Bifidobacterium	*Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis* subsp. *lactis*

strain from production to storage and distribution processes, and only 5 genera and 19 species of microbes were approved as probiotic strains by the food code published by MFDS (Table 3).

International standards for probiotics, established by the WHO, FAO, and EFSA, mainly focus on the safety of strains. To ensure safety, genotypic safety related to antibiotic tolerance, virulence factors, genome stability, and phenotypic safety such as antibiotic resistance, toxic compounds production, and hemolytic activities should be evaluated [20–22]. In addition, the origin and microbial characteristics, as well as acute oral toxicity evaluation in animal models, should be evaluated [23].

As mentioned above, probiotic strains should also exhibit probiotic properties. Maintaining their metabolic activities in simulated environments in the gastrointestinal tract, such as acids, enzymes, and bile salts, should be investigated [24, 25]. In addition, gut colonization abilities that are competitive with beneficial or harmful microbes needed to be evaluated to show probiotic poten-

Table 2. General prerequisites of probiotics.

Standard	Species
Safety	1. Management of classification history - Identification at genus, species, and strain levels - Glycolysis, enzyme activity, 16s rRNA sequencing, etc. 2. Phenotype (non-pathogenic) - Antibiotic resistance, toxic production, hemolytic activity 3. Genotype - Absence of antibiotic tolerance properties, absence of pathogenic and toxicities, and absence of metastatic potentiality 4. Safety evaluation of animal models with weak immunity 5. History of safe use - Serious side effects
Functionality	1. Survival and metabolic activities in the gastrointestinal (GI) tract - Resistance to bile salts and enzymes - Resistance to low pH in stomach 2. Competitiveness for microbial species living in GI tract - Antagonism to pathogens - Production of endogenous gut microbiomes and resistance to antibiotic and acid conditions - Adhesive ability for gut colonization 3. Other proven health function improvement effects (individual strains may have difference)
Stability	1. High productivity through high-efficiency culture - Ensuring genetic stability 2. Survival rate in freeze-drying, raw material preparation, and distribution process after incubation 3. High storage survival rate of final products - Moisture activity, temperature, pH, oxidation, osmotic pressure, etc. 4. Maintaining sensorial characteristics of final products

Table 3. Legally approved probiotics as health functional foods by the Ministry of Food and Drug Safety in the Republic of Korea.

Genus	Species
Lactobacillus	*Lactobacillus acidophilus, Lactobacillus gasseri, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus rhamnosus, Lactobacillus fermentum, Lactobacillus reuteri, Lactobacillus plantarum, Lactobacillus salivarius*
Lactococcus	*Lactococcus lactis*
Enterococcus	*Enterococcus faecium, Enterococcus faecalis*
Streptococcus	*Streptococcus thermophilus*
Bifidobacterium	*Bifidobacterium bifidum, Bifidobacterium animalis* subsp. *lactis, Bifidobacterium breve, Bifidobacterium longum*

tials [26, 27]. Thus, other health functions such as cholesterol assimilation, nitrile-depleting, antibacterial, antioxidative and immunomodulatory may have been dealt with [28].

Finally, to improve shelf life from the process-point of view the strains should have high productivity and high stability (or high survivability) under harsh processing conditions such as freeze-drying, spray-drying, and distribution steps [29]. In addition, it would be beneficial if the strains could be fermented in high mass per unit volume or if the products could be served for longer storage periods with good viability [30, 31].

Functionalities of Probiotics

Based on the standards of health functional foods, any products using these 19 species approved as probiotic strains by the MFDS in the Republic of Korea must be prepared by culturing microorganisms in a safe (food-grade) medium with protective agents or by drying cultured microorganisms or their mixtures. In addition, when culturing the strains. Additionally, probiotic products are recognized for their ability to promote the proliferation of beneficial bacteria suppress harmful bacteria, improve bowel activities and intestinal health are fundamental for probiotic strains [32]. Accordingly, the most common functionality of probiotics is to prevent various diseases by stabilizing the intestinal microbiome, inhibiting constipation, and reducing the production of decomposed products [33, 34]. In addition to the intestinal health-related functions, immune function, cholesterol improvement, antioxidant, fatigue improvement, body fat control, joint/bone health, sleep quality improvement, skin health, blood pressure control, blood flow improvement, memory improvement, liver health, eye health, relaxation, cognitive improvement, prostate health, calcium absorption, urinary tract health, dental health, muscle improvement, men in andropause, women in menopause, improvement of immune sensitive skin, urination function, gastric health/digestion function, sperm motility, the proliferation of beneficial bacteria in the vagina, suppression of harmful bacteria, improvement of the premenstrual condition, and growth of children would become targets of health benefits by probiotics [35–43].

The potential applications of probiotics as functional food supplements have been widely studied (Table 4). Various types of *Lactobacillus* have exerted beneficial effects on different symptoms. For example, *L. rhamnosus* has been used to treat acute infectious diarrhea, atopic dermatitis, food allergies, cancers such as colon cancer, and inflammation. In addition, *L. acidophilus* and *L. plantarum* showed positive effects on intestinal diseases, such as diarrhea and irritable bowel disorder and on obesity such as reduced cholesterol levels. *Bifidobacterium* help to relieve atopic dermatitis and type 2 diabetes and to decrease obesity, immunomodulation, irritable bowel syndrome, and lactose intolerance levels. *Streptococcus* can be used for intestinal, oral health, and as an antioxidant agent. Furthermore, *Enterococcus* are helpful immune modulation.

Although many studies have been performed, legal approval for using probiotics as health functional foods is difficult to obtain because probiotic safety and functionality should be scientifically proven. "Notification of Standards and Specifications of Health Functional Foods" in the MFDS only approves specific strain with functionality. They are individually registered and managed as health functional components (i.e., standards of probiotics), and most of the raw ingredients are classified as physiological functions at levels 2 and 3 (Table 5). Level 2 means 'physiological function', which can be given if the product has a special effect on the normal function or biological activity of the human body, so it has an improvement or maintenance of human health. Level 3 is 'function that decreases the risk of occurrence

Table 4. Health benefits of probiotics.

Effect	Bacterium	Reference
Acute infectious diarrhea	*L. rhamnosus* GG, *L. reuteri* R2LC, *L. acidophilus* NDCO 1748, *E. faecium* SF 68	Marteau PR, de Vrese M, Cellier CJ, et al, *Am. J. Clin. Nutr.* 73, 430S-436S (2001).
Irritable bowel syndrome (IBS)	*B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. plantarum, S. thermophilus*	Kim HJ, Vazquez Roque MI, Camilleri M, et al, *Neurogastroenterol. Moti.,* 17, 687-696 (2005).
Inflammatory Bowel Disorder (IBD)	*L. plantarum* 299V	Schultz M and Sartor RB, *Am. J. Gastroenterol,* 95, S19-S21 (2000).
Atopic dermatitis and food allergies	*L. rhamnosus* GG, *B. lactis, L. paracasei, L. reuteri, B. longum*	Majamaa H, Isolauri E, *J. Allergy Clin. Immunol,* 99, 179-185 (1997).
Cholesterol levels reduction	*L. acidophilus* NCFM	Percival M, *Clin. Nutr. Insights,* 6, 1-4 (1997).
Helicobacter pylori infections	*L. salivarius*	Aiba Y, Suzuki N, Kabir AM, et al, *Am. J. Gastroenterol,* 93, 2097-2101 (1998).
Oral health	*S. thermophilus* NCC 1561, *L. lactis* NCC2211	Comelli EM, Guggenheim B, Stingele F, et al, *Eur. J. Oral. Sci,* 110, 218-224 (2002).
Colon cancer	*L. acidophilus, L. rhamnosus* GG	Gorbach SL, Goldin BR, *Nutr. Rev,* 50, 378-381 (1992).
Type 2 diabetes	*L. helveticus, S. cerevisiae, B. lactis* Bb12, *L. acidophilus* La5	Akbari V and Hendijani F, *Nutr. Rev,* 74, 774-784 (2016).
Obesity	*L. curvatus* HY7601, *L. plantarum* KY1032, *L. paracasei* CNCM I-4270, *B. breve* CNCM I-4035	Kobyliak N, Conte C, Cammarota G et al, *Nutr. Metab,* 13, 1-13 (2016).
Immunomodulation	*L. plantarum* 299v, *B. bifidum* MF 20/5, *L. casei* W56, *E. faecium* SF68	Lomax A and Calder P, *Curr. Pharm. Des,* 15, 1428-1518 (2009).
Lactose intolerance	*B. longum* Bifina, *L. reuteri* Reuterin	Oak SJ and Jha R, *Crit. Rev. Food Sci. Nutr,* 59, 1675-1683 (2019).
Anti-cancer, anti-inflammatory effects, enhancement of gut barrier	*L. casei* ATCC 334, *L. rhamnosus* GG	Escamilla J, Lane MA, and Maitin V, *Nutr. Cancer,* 64, 871-8 (2012).
Anti-adhesion effect against *E. coli*	*Lactobacillus* spp.	Abbas HH, Abudulhadi S, Mohammed A, et al, *Int. J. Adv. Res,* 4, 614-620 (2016).
Immunomodulatory activity	*L. rhamnosus* GR-l, *L. paracasei, L. gasseri, L. helveticus, L. reuteri, B. coagulans, B. bifidum, S. thermophilus*	Koscik RJ, Reid G, Kim SO, et al, *Reprod. Sci*, 25, 239-245 (2018).
Protective effect against the *E. coli*	*L. rhamnosus* GG	He X, Zeng Q, Puthiyakunnon S, et al, *Sci. Rep*, 7, 43305 (2017).
Suppression of multidrug-resistant *Helicobacter pylori*	*Lactobacillus isolates*	Lv C, Jia F, Wang D, et al, *Jundishapur J. Microbiol,* 12, e91797 (2019).
Cholesterol-lowering and immunomodulatory effect	*L. bulgaricus* ATCC 11842,	Tok E and Aslim B, *AMB Express* 2, 66 (2010).
Anti-biofilm and anti-microbial effects against	*L. jensenii, L. gasseri, L. fermentum, L. plantarum*	Wang K, Niu M, Song D, et al, *J. Biosci. Bioeng,* 129, 206-214 (2019).
Anti-aging	*L. paracasei*	Wang S, Ahmadi S, Nagpal R, et al, *GeroScience* 42, 333-352 (2020).
Lowered the intestinal epithelial apoptosis	*L. acidophilus*	Guo Y, Jiang X, Yang Y, et al, *J. Func. Foods,* 47, 91-99 (2018).
Antioxidant	*S. thermophilus, L. bulgaricus, L. jonsonnii, L. acidophilus*	Aguilar-Toalá, JE, Garcia-Varela R, Garcia HS, et al, *Trends Food Sci. Technol,* 75, 105-114 (2018).

Table 5. Probiotics and lactic acid bacteria as health functional ingredients (individual standards).

Name (individual standard number)	Company	Functionality
Probiotics DeSimone (2009-28)	Bioeleven Co., Ltd.	- Proliferation of beneficial bacteria, control of harmful bacteria, facilitation of bowel movement - It can help intestinal health by controlling gut immunity (physiological functions level 2)
L. plantarum CJLP133 (2013-11)	CJ CheilJedang Co., Ltd.	- It can help improve skin condition by immune overeaction (physiological functions level 2)
L. sakei Probio65 (2013-17)	Probionic Co., Ltd.	- It can help improve skin condition by immune overeaction (physiological functions level 2)
L. gasseri BNR17 (2014-5)	Bioneer Co., Ltd.	- It can help reduce body fat (physiological functions level 2)
Probiotics ATP (2014-16)	Cellbiotech plant 1,2	- It can help improve skin condition by immune overreaction, but the related human application test is insufficient (physiological functions level 3)
UREX probiotics (2014-27)	Vixxol Co., Ltd.	- It can help women's vaginal health through proliferation of lactic acid bacteria (physiological functions level 2)
L. plantarum HY7714 (2015-1)	HY Co., Ltd. Pyeongtaek Probiotics plant	- It can help moisturize human skin (physiological functions level 2) - It can help maintain skin health from UV (physiological functions level 2)
L. gasseri BNR17 (2017-6)	AceBiome Co., Ltd.	- It can help reduce body fat (physiological functions level 2)
L. rhamnosus IDCC 3201 (2018-12)	Il Dong Pharmaceutical Co., Ltd.	- It can help improve skin condition by immune overreaction (physiological functions)
Lactobacillus mixture HY761 + KY1032 (2019-4)	HY Co., Ltd.	- It can help reduce body fat
L. acidophilus YTI (HU038) (2019-22)	Huons Natural Co., Ltd.	- It can help women's health during menopause
Respecta probiotics (2019-26)	COSMAX NS Co., Ltd.	- Proliferation of beneficial bacteria and control of harmful bacteria in the vagina
L. plantarum IN75 and *B. longum* IM55 mixture (NVP1703) (2019-28)	Navipharm Co., Ltd.	- It can help improve nasal conditions by immune overreaction

of a disease', and the level of scientific based data secured is high to meet Significant Scientific Agreement [44]. After the first approval of the probiotic complex using eight different probiotic strains for intestinal health function in 2009, a total of 13 probiotic strains products were listed in individual standards. *L. plantarum* CJLP133, *L. sakei* Probio65, Probiotics ATP, *L. plantarum* HY7714, and *L. rhamnosus* IDCC 3201 help improve skin conditions through inhibiting immune overreaction. Both *L. gasseri* BNR17 and *Lactobacillus* HY761/KY1032 reduce body fat, and UREX and Respecta probiotics improve women's vaginal health. *L. acidophilus* YTI help women's health during menopause, and mixtures of *L. plantarum* IN75 and *B. longum* IM55 improve nasal health.

Research for Next Generation Probiotics

Evidence for the role of probiotics in maintaining human health is mainly colonization and communication of probiotics in the human gut mucosa [45]. These are highly dependent on the properties of individual strains and affected by their environment, such as microbiota, pH of gastrointestinal tract, consumed food sources, and bioactive compounds [46]. Thus, future probiotic studies should be focused on inter- or intra- relationships, which might be adjusted to each person [47].

Prebiotics, postbiotics, metabiotics, parabiotics

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" based on ISAPP [48]. Oligosaccharides, including inulin, galacto-, fructo-, and xylo- oligosaccharides, and lactulose are common prebiotics [49]. Recently, non-digestible ingredients (by themselves or food) are also considered prebiotics because they stimulate probiotic growth, affect intestinal conditions, and are used as a medium in the intestines without being easily hydrolyzed. Accordingly, combinations of probiotics and prebiotics are called synbiotics [50].

The postbiotics, metabiotics, and parabiotics are emerging concepts in the probiotics industry because of their health beneficial properties. However, they are not well defined yet and are closely related to each other [51]. Postbiotics are defined as 'the preparation of inanimate microorganisms and/or their components that confer health benefits to the host' [50]. Recently, postbiotics have been limited to cell-free supernatants and soluble factors including metabolites secreted by probiotics such as enzymes, proteins, short-chain fatty acids, organic acids, and bacteriocin [52]. Metabiotics are referred to as cell-free supernatants, postbiotics, or biogenics. They are defined as 'structural components of probiotics and/or their metabolites, including signaling molecules with known chemical structures'. Metabolites may be safe and well-dosed because of their exact chemical structure [53]. Parabiotics refer to 'inactivated or ruptured probiotic cells or cell extracts containing cell components' [51]. Overall, postbiotics, metabiotics, and parabiotics can be described as follows: a complex mixture of metabolic products mainly from supernatants (postbiotics), structural chemicals (metabiotics), or inactivated cell/cell components (parabiotics). However, all biotics originate from probiotics and are beneficial to human health. In addition, they can be obtained in various forms depending on the probiotics strains, implying different metabolic functions that promote health.

Gut microbiome

The era of post-genome has arrived with the completion of the human genome project. In particular, humans have twice as many microorganisms and 100 times more microbial genes than human cells, and thus human genes cannot be studied properly without those of microorganisms [54, 55]. The microbiome is close enough to a human genome to be called a second genome or a second brain, and it is known that various diseases can be caused by imbalances in the gut microbiome [56]. Therefore, microbiome research can analyze the correlation between the gut microbiome and disease, using this association to develop new medicines and identify the cause of diseases. The gut microbiome consists of beneficial, harmful, and intermediate bacteria. Beneficial bacteria can control hormone secretion in the human body; thereby, stimulating the immune system and suppressing intestinal diseases or pathogens activation [57–59]. Harmful bacteria can produce endotoxins, causing various autoimmune diseases, such as inflammation or asthma, chronic or seasonal allergies, atopic allergies, and rheumatism [60]. Thus, imbalance between beneficial and harmful bacteria in intestine frequently leads to considerable damage to the immune system [61]. Therefore, several studies have focused on the maintenance of human health through microbiome research such as whole genome sequencing of microorganisms living in the human body [62, 63].

Probiotics can regulate gut barrier function, immunomodulation, and colonization resistance against pathogens [64]. More specifically, probiotics change composition of gut microbiota, and thereby induce changes of gut microbial community, resulting in production of beneficial metabolites such as short-chain fatty acids (SCFAs) [65, 66]. SCFAs (e.g., acetic acid) typically induce acidic intestinal environment, suppressing the colonization of pathogens and improving dietary absorption [67]. In addition, SCFAs improve the bowel movement by strengthening peristalsis [67].

A communication occurs between individual bacterium and host cell within microbiome [68], particularly in GI tract [69]. Individual cell typically recognizes and respond to host signals by secreting various signal molecules, which can continuously interact with host in brain, nervous system, and immune system [70, 71]. Various substances from postbiotics, metabiotics, and parabiotics include different classes of metabolites, peptides, biogenic amines, and short-chain fatty acids and those can be potentially utilized as signaling molecules. As an example of communication between microbiota and host cells, psychobiotics, which is probiotics to release neuroactive substances that directly affect the brain, mind, and behavior of humans, were introduced to improve the balance of the gut-brain axis [72]. Recent reviews also have addressed association between microbiota and host in terms of fecal transplantation containing probiotics and intestinal microorganisms [71, 72].

Previous studies showed the correlation between *Bifidobacterium* spp. and single nucleotide polymorphism (SNPs). To find alleles that coexist for different hosts with microbiome properties is one of the feasible ways to investigate potential host-microbiota interactions. The most representative example is that a child who has a lactase non-persistence have a lactase persister when becoming adult, probably because of genetic changes. Lactase non-persistence, or lactase intolerance, indicated the low level of lactase genes, resulting in low absorption of lactose because lactose cannot be hydrolyzed into D-glucose and D-galactose. Specifically, infants intake breast milk and have a lactase non-persistence. Accordingly, levels of the lactase gene and the abundance of *Bifidobacterium* spp. were found to be inversely proportional. It was revealed that some babies with lactase-persistence had low intestinal abundance of *Bifidobacterium* spp. This is due to the association between *Bifidobacterium* spp. and single nucleotide polymorphism (SNPs) near the lactase gene, which is most consistent among signals in the gut microbiome [73, 74].

Gut-brain axis

Professor Michael Gershon, a Columbia neurobiologist, used the term "The second Brain" at first by revealing that the brain does not care about digestion. The intestine has more than 100 million neurons and can directly govern digestion activity, such as intestinal movement and enzyme secretion, because the brain must perform many functions in the body [73]. Patients with irritable bowel syndrome (IBS) and Crohn's disease (CD) caused by imbalance of the gut microbiome, have malfunction of stress-regulating hormones [76]. Based on the involvement of microbiomes in health and disease, as well as the microbial diversity and its various functions, the effects of the genetic factors of humans on microbiome composition had been investigated, since genes may give beneficial health effects by promoting stable microbial communities in the intestine. The study of heritability yielded a consistent subset of microbes affected by the genes; however, it was difficult to identify specific genetic variants associated with microbial phenotypes through genome-wide association studies (GWAS). In particular, the technical hurdles of GWAS were attributed by the necessity of reducing the burdens of multiple tests and difficulties of processing microbiome datasets [73]. Therefore, studies conducted so far are limited by the GWAS, and comparison and verification among studies become more important to identify authentic signals.

To date, several consistent associations that can predict disease sensitivity, particularly by quantifying the microbiome's genotype, environment, and interactions between the microbiome have been suggested [61, 75]. For instance, unhealthy intestinal conditions can worsen many brain diseases such as depression, dementia, and autism [77]. As another example, probiotics can help control negative emotions, such as fear and anxiety, by producing the neurotransmitter γ-aminobutyric acid (GABA) and a happiness neurotransmitter called serotonin [78]. According to a previous study, depression in patients had improved after ingestion of probiotics, and autistic children had increased language skills after probiotics consumption.

The gut has as many neurons as the brain, and thus it signals the intestinal nerve cells to promote neurotransmitters. This means that the condition of the intestinal microbiome can affect brain function. In 2004, Nobuyuki Sudo's research team at Kyushu University in Japan studied the effect of gut microbiome on brain-changes in mice [79]. When the mice were in a small container to be exposed to stress, the mice without a gut microbiota had higher levels of a stress hormone called corticosterone than normal control mice. Interestingly, when transplantation of beneficial *Bifidobacterium* spp. was conducted in mice without the gut microbiota, this exaggerated-stress response could be normalized. In contrast, administration of the harmful bacterium such as enteropathogenic *E. coli* could not recover the stress response. In addition, when aseptic mice were 6 weeks old, the stress response was normalized when feces were transplanted from normal mice, but the stress response did not recover when transplanted at 8 or 14 weeks of age. As a result, it was found that beneficial bacteria are needed to develop brain functions, such as response to stress, at an adequate age. Subsequent studies have shown that gut microbiomes are related to various brain activities, such as the development of the blood-brain-barrier (BBB), nervous system formation, production of the brain's immune cells called microglia, and myelination of neurons [72, 80].

Fecal transplantation

Fecal transplantation means the administration of feces from an individual into another intestine. In the case of human, it has been implemented in a way that the gut microbiome from the feces of healthy people is purified and put into the patient's intestines [81]. Initially, it was injected with an colonoscopy however, recently, it is administered as a capsule-type pill [82]. Fecal transplantation has been used to treat autism, obesity-related eating habits, and even chronic inflammatory bowel diseases. Fecal transplantation for the treatment of chronic inflammatory bowel disease was first studied by bacteriologist Stanley Falkow in 1957. He fed feces from healthy people to the patients to prevent the destruction of the normal gut microbiome by the excessive use of antibiotics. However, this study was not acceptable at that time, and it could not be published despite positive results of fecal transplantation [83]. In 1958, Eiseman, a Colorado surgeon, conducted a clinical trial on a patient to restore the normal gut microbiome, confirming a similar positive effect [84]. Specifically, four patients with gastritis, who did not respond effectively to existing treatment, were treated using the feces of healthy people. The fecal transplantation showed a success rate of appproximately 94%. Since then, the causes of gastritis are the destruction of gut microbiomes and activation of *Clostridium difficile* owing to the excessive use of antibiotics. Subsequently, vancomycin was administered to remove the causative agent. In the early 1990s, Professor Khoruts of Minnesota Medicine School conducted fecal transplantation. A 64-year-old female patient with chronic inflammatory bowel disease, who could not be treated with antibiotics for eight months at another hospital, was injected with the feces of her husband mixed with physiological saline into her intestine through a colonoscopy. As a result, she was able to maintain normal bowel movements after fecal transplantation by stopping continuous diarrhea [85]. Fecal transplantation for chronic inflammatory bowel disease has shown a positive therapeutic effect of approximately 90% [86].

Conclusion

Currently, the demand for probiotics will increase owing to high health concerns caused by viral disease, such as MERS and COVID-19, an aging society, and easy access to health knowledge. Next generation probiotics are emerging for whole-body health as well as existing functionalities for the intestine. In the future, customized probiotics will be proposed by collecting different gut microbiome compositions from each person. Microbiome research can be developed in combination with biotechnologies such as synthetic biology, and biomaterial engineering. In addition, multi-omics network analysis can be used to study the functional correlation of gut microorganisms in a specific environment and generate a database. Therefore, studies on probiotic functionalities using various biotechnologies will be further developed to help improve people's health.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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