Next-Generation Probiotics- The Future of Biotherapeutics

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Abstract

Lactic acid bacteria, most of which are represented by the genera *Lactobacilli* and *Bifidobacteria*, have been extensively investigated for their role as probiotics and have also been used in food items due to their advantageous metabolic properties in manufacturing fermented food. To expand the spectrum of probiotics, there is currently a great deal of interest in researching different microorganisms with potential health benefits for humans. These next-generation probiotics are bacteria that mostly come from the *Bacteroides*, *Clostridium*, *Faecalibacterium*, and *Akkermansia* genera. However, studying these microbes as probiotics and using them in food production can become extremely problematic. Understanding its efficacy and safety for consumption, as well as its application in the production of new food items and industrial-scale food production, are among some of the challenges faced. The next generation of probiotics has become a major topic in scientific research as well as the food sector and poses new obstacles as it gets studied further.

Keywords: Next-generation probiotics (NGP); Functionality; Biologics; Therapeutics.

Abbreviations: NGP, Next generation probiotic; FAO, Food and Agriculture Organization; WHO, World Health Organization; LAB, Lactic Acid Bacteria; TMAO, Trimethylamine-N-oxide; GABA, Gamma-aminobutyric acid; SCFAs, Short-chain fatty acids; NGS, Next Generation Sequencing; PSA, Capsular polysaccharide A; BMI, Body Mass Index; VBNC, Viable but non-culturable; FDA, Food and Drug Administration; US-LBP, United States FDA's program on Live Biotherapeutic Products.

Significance | Probiotics as potential biotherapeutic agents

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Edited by Mst Murshida Mahbub, PhD, East West University, Dhaka, Bangladesh, and accepted by the Editorial Board May 30, 2022 (Received for review April 9, 2022)

Introduction

The human microbiota, which consists of 100 trillion bacteria, forms a symbiotic connection with the host and plays a significant role in health promotion as well as the start and progression of illnesses (Bron et al., 2017; Ferdous et al., 2018). Intestinal tract microbes take part in biological processes that regulate metabolic phenotype, and development of the epithelial lining, and also influence innate immunity. Furthermore, Intestinal dysbiosis (alteration of intestinal microbiota composition) has the potential to develop complex diseases like diabetes mellitus, obesity, asthma, neurodegenerative diseases, and inflammatory bowel disease, among others (Levy et al., 2017). The identification of beneficial bacterial strains capable of treating intestinal dysbiosis and hence promoting health has resulted from a thorough understanding of the intestinal microbiota (Adak and Khan, 2019).

The term "probiotics" is now commonly used not just by medical experts, but also by the general public outside of medicine, as a synonym for "a method of enhancing one's health." Interestingly, probiotics are frequently used to refer to both medications and foodstuffs. Probiotics, on the other hand, are defined by the joint FAO/WHO group as "living microorganisms that, when administered into the body in sufficient proportions, offer a health benefit" (Hill et al., 2014).

The majority of microorganisms licensed and sold as probiotics today belong to the lactic acid bacteria (LAB) group, which is mostly represented by the genus *Lactobacilli* (Brodmann et al., 2017).

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Please cite this article:
Fakruddin M, Shishir MA, Yousuf Z and Khan SS (2022). Next Generation Probiotics- The Future of Biotherapeutics. *Microbial Bioactives*, 5(*1*), 156-163.

They are metabolically distinguished by the production of lactic acid from carbohydrates, resulting in an acidic environment that prevents the development of some pathogenic bacteria species (Chowdhury et al., 2012; Fakruddin et al., 2017). They may also generate secondary metabolites such as bacteriocins, exopolysaccharides, and enzymes, all of which are beneficial to human health (Chowdhury et al., 2013; Heinken et al., 2014).

Despite the benefits mentioned above, current probiotic development trends seek to reduce the usage of probiotic groups like *Lactobacillus* and enhance the use of other genera and species of bacteria that are more suited to the intestinal environment (Fernández-Murga & Sanz, 2016; Anvar & Nowruzi, 2021). These bacteria are termed Next Generation probiotics (NGPs). The growing popularity of NGPs in recent years can be linked to the various benefits they provide over conventional probiotics (Shyam et al., 2021; Han et al., 2021; Lopez-Moreno et al., 2021). In-depth research into this new generation of probiotics will allow for the development of more targeted tools to aid in the treatment of emerging disorders (O'Toole et al., 2017; Sun et al., 2019).

The goal of this review is to provide an overview of some of the prospective next-generation probiotics and their health benefits.

Health benefits of probiotics

Interest in the use of probiotics in formerly undiscovered areas of application has grown as a result of the increasing amount of gut microbiota research throughout the world. Probiotics used in medicine have nearly solely dominated *Lactobacilli* strains as being the most promising both scientifically and commercially, for decades. Probiotics' beneficial impacts on microorganisms have been researched extensively; they "activate" first at the local level (for example, in the intestinal lumen, on the mucous membrane, and in the submucosal layer) and subsequently at the system level. The components of probiotics' cell walls (peptidoglycans, teichoic acids, capsular polysaccharides) and their expressed proteins mediate their effects on organisms. Due to direct antagonism (inhibition), competitive restriction of receptor binding, indirect effects on commensal microorganisms, and competition for nutritional molecules, probiotics have been found to reduce enteropathogens' access to intestinal epithelial cells (table-1).

Next-Generation Probiotics (NGPs)

Lactobacillus spp. and other commonly used probiotics were picked "at random" - based on research of people's dietary patterns. Although most of these probiotics are biologically safe and some are clinically effective, the effects of their usage are statistically insignificant in the context of evidence-based medicine. Furthermore, traditional probiotics are also not utilized to treat certain disorders (Bottacini et al., 2017). As a result, the identification and practical use of more potent and disease-specific next-generation probiotics is a critical research area at this moment across the world.

Many previously unrecognized probiotic bacterial strains have been identified from intestinal microbiota using modern next-generation sequencing techniques, and these next-generation probiotics have become potential sources for novel drugs as therapeutics for a variety of diseases, including gastroenterological pathology (Chang et al., 2019). When compared to regular probiotics, new-generation probiotics have several benefits (Table-2). Numerous metabolites (such as folate, indoles, secondary bile acids, trimethylamine-N-oxide (TMAO), serotonin, gamma-aminobutyric acid (GABA), short-chain fatty acids (SCFAs)—acetate, propionate, butyrate, and others) have been discovered from NGPs to date, all of which can play a role in the regulation of physiological host phenotype (Husted et al., 2017; Kimura et al., 2014; Byndloss et al., 2017; Clarke et al., 2014; Olveira et al., 2016).

Candidates for Next Generation Probiotics

Bifidobacterium spp.

Bifidobacterium spp. is a member of the *Actinobacteria* phylum. *Bifidobacterium* spp. strains have been shown to be effective in the treatment of colitis (inflammation of colon) (Lin et al., 2019). Anticancer treatment has shown that *B. breve* and *B. longum* are effective against colorectal cancer, and that these strains can improve the impact of anti-cytokine preparations (Sivan et al., 2015). After hepatocellular carcinoma resection, body responsiveness to *B. longum* and *Enterococcus hirae* induces a persistent CD8+-T-cell response and improves prognosis (Rong et al., 2017). *Bifidobacterium* spp. (especially *Bifidobacterium longum*) can enter the circulation and specifically accumulate in malignant tumors (Li et al., 2010).

Akkermansia muciniphila

The Verrucomicrobia phylum includes *Akkermansia muciniphila*. One of *A. muciniphila's* most notable characteristics is its capacity to use intestinal mucins, glycoproteins from the epithelial mucus layer, as its only source of carbon and nitrogen (Derrien et al., 2004; Reunanen et al., 2015). *A. muciniphila* protects against type 2 diabetes and obesity via modulating the endocannabinoid system (neuromodulatory system), which regulates glucose metabolism (Cani and de Vos, 2017). It has been discovered that prebiotics of the insulin type raise the amount of *A. muciniphila*, which helps to enhance metabolism in obese people (Everard et al., 2013). Anti-cytokine medications used in cancer have also been demonstrated to be enhanced by *A. muciniphila* in model animals (Schneeberger et al., 2015). Patients with inflammatory bowel diseases and metabolic disorders were found to have lower levels of *A. muciniphila*, suggesting that this bacterium may have anti-inflammatory characteristics (Derrien et al., 2017; Collado et al., 2007). The capacity

Table 1| **Effects of probiotics on the body** (Pesce et al., 2022; He et al., 2021; Lin et al., 2019)

Immune	Non-immune
Increased production of secretory immunoglobulin A	Stimulation of protective products for mucus epithelium
Correction of local and systemic cytokine profile	Decreased permeability of the intestinal epithelium
Activation of antigen-presenting local macrophages	Deletion of bacteriocins that suppress intestinal pathogens
Reduced induction of food antigens	Inactivation of toxins produced by intestinal pathogens

Table 2| **Comparison between traditional probiotics and next-generation probiotics** (Kazmierczak-Siedlecka et al., 2022; Torp et al., 2022; Yang et al., 2021; Cunningham et al., 2021)

Traditional probiotics	Next-generation probiotics
Identified from life experiences	Identified from bioinformatics and/or NGS studies
Considered safe for human	Safety needs to be evaluated
Mode of action not well defined	Well-defined mode of actions
The limited spectrum of microbial genera and species	The wide spectrum of microbial genera and species
Target general sub-health population	Target specific diseases
Mostly used as a supplement	Pose potential to be used as biotherapeutics

of *A. muciniphila* to aid the repair of the compromised intestinal barrier caused by a high-fat diet explains these positive benefits (Everard et al., 2013).

Bacteroides fragilis

Bacteroides fragilis belongs to the Bacteroides species. The *B. fragilis* strains that lack the enterotoxin gene, display many major benefits (Round and Mazmanian, 2010; Goloshchapov et al., 2020). The capsular polysaccharide *B. fragilis* (PSA), which can modulate microbiota-host interactions, plays the most important function in this. PSA increases T-cell anti-inflammatory memory thanks to zwitterionic motifs, which lowers systemic inflammation symptoms (Lukiw, 2016; Tan et al., 2020; Nowruzi et al., 2022). Because of their potential to reduce pathogen-induced inflammation and cure autism spectrum disorders, as well as their ability to reduce *Vibrio* infections and antibiotic-induced diarrhea, *B. fragilis* strains are regarded as promising next-generation probiotics (Zhang et al., 2018).

Christensenella minuta

Christensenella minuta belongs to the Firmicutes family and has been shown to have probiotic benefits in the treatment of obesity and related metabolic diseases. Individuals with a low BMI have a high amount of Christensenellaceae (Goodrich et al., 2016). It has also been proven that the usage of *C. minuta* may enhance the microbiota related with obesity (Goodrich et al., 2014) and it's also been discovered that taking *C. minuta* increases the formation of SCFAs (short-chain fatty acids) (Breton et al., 2022).

Faecalibacterium prausnitzii

Faecalibacterium prausnitzii is one of the most promising bacteria for developing next-generation probiotics at present. The Ruminococcaceae family includes *F. prausnitzii* (Carlsson et al., 2013). These bacteria can ferment glucose to produce SCFA (butyrate, formic acid, and D-lactate), allowing the gut to remain in a state of homeostasis (Miquel et al., 2013). Bacteria belonging to the genus *Faecalibacterium* are being studied as a modulator in cancer immunotherapy (Ferreira-Halder et al., 2017). Scientists have found a link between the quantity of *Faecalibacterium* bacteria in the intestines and the long-term survival of melanoma patients. Furthermore, it also reveals that the number of regulatory T cells and the level of pro-inflammatory cytokines IL-6, IL-8, and soluble IL-2 receptor in the blood during melanoma metastases were adversely linked with the concentration of *Faecalibacterium* in the colon (Chaput et al., 2017). The findings show that *F. prausnitzii* is an important therapeutic target as well as a prognostic marker in cancer patients (Gopalakrishnan et al., 2018). The butyrate generated by carbohydrate fermentation has been linked to *F. prausnitzii's* health advantages, which include an immunomodulatory response in the host, improved intestinal barrier integrity, and anti-inflammatory effects (Saarela, 2019). *F. prausnitzii*, on the other hand, has the power to interact with the host's health through immunomodulatory, energy-producing, and anti-inflammatory actions, and can also act as a diagnostic marker in a variety of diseases, including parkinson's disease, alzheimer's disease, depression, type 2 diabetes mellitus, crohn's disease, and irritable bowel syndrome (Zhang et al., 2014).

Prevotella copri

The Bacteroidetes phylum contains *Prevotella copri.* This microbe's probiotic strain can improve glucose tolerance and liver glycogen levels (De Vadder et al., 2016). *P. copri* has been recommended as a possible target for metabolic illnesses including type-2 diabetes and obesity (Lin et al., 2019).

Parabacteroides goldsteinii

Parabacteroides goldsteinii is considered as a next generation probiotic for obesity (Chang et al., 2015). *P. goldsteinii* levels are much lower in the microbiota of mice fed with a high-fat diet, but are significantly higher after prebiotic polysaccharide therapy. It aids in weight reduction when paired with *P. goldsteinii* prebiotics, along with increasing the permeability of the intestines, metabolic endotoxemia, inflammation, and insulin resistance (Wu et al., 2019). *P. goldsteinii* has also been shown to have anti-inflammatory and insulin-stimulating properties (Lin et al., 2019).

Clostridium butyricum

Clostridium butyricum is a Gram-positive, spore-forming, and obligate anaerobe, meaning it is extremely sensitive to oxygen. Because of its capacity to generate significant amounts of butyric acid, the microbe is given the name '*butyricum*' (Cassir et al., 2016). The capability of this species of *Clostridium* to ferment non-carbohydrates digestible, creating short-chain fatty acids (mostly butyric acid), which are a primary basis for enterocyte proliferation and play an important role in maintaining colonic health (Hamer et al., 2008; Sun et al., 2016). *C. butyricum* has been discovered to greatly reduce the formation of intestinal tumors in mice induced by a high-fat diet in the prevention and treatment of cancer. In addition, the bacteria cause intestinal tumor cells to proliferate less and undergo more apoptosis (Chen et al., 2020). Finally, therapy with *C. butyricum* strains in conjunction with antidepressants resulted in considerable improvement in depression in depressed individuals (Miyaoka et al., 2018).

Eubacterium hallii

E. hallii is a Gram-positive, catalase-negative anaerobic bacterium belonging to the family Lachnospiraceae of the phylum Firmicutes (Duncan et al., 2004). *E. hallii* is a species that can generate butyrate by fermenting carbohydrates. Its capacity to manufacture butyrate utilizing both lactate and acetate as a substrate of metabolism distinguishes it from other genera of bacteria of intestinal origin (Louis et al., 2009). *E. hallii* has been characterized as a crucial species within the intestinal food chain that has the ability to have a significant influence on metabolic balance as well as the gut microbiota by forming various short-chain fatty acids from dietary polysaccharides or the host itself (Engels et al., 2016). It was shown that daily oral dose of *E. hallii* improves insulin sensitivity and boosts metabolic energy in obese and diabetic mice. Furthermore, increasing dosages of *E. hallii* had no effect on treated mice's body weight or food intake, suggesting that the bacteria might be a novel, safe and effective probiotic strain for improving insulin sensitivity in the treatment of obesity and diabetes (Udayappan et al., 2016). *Bifidobacteria*, which are naturally found in the gut microbiota and in breast milk, may digest complex carbohydrates to produce monosaccharides, which can then be utilized by *E. hallii* to produce short-chain fatty acids (Bunesova et al., 2018). This symbiotic relationship between *Bifidobacteria* and *E. hallii* suggests a significant and advantageous relationship for the host.

Other emerging probiotics

Probiotic bacteria such as *Eubacterium limosum*, *Enterococcus hirae*, *Enterococcus faecium*, *Collinsella aerofaciens*, and *Burkholderia cepacia*, which were previously unknown, were shown to improve the efficacy of anti-tumor immunotherapy (Routy et al., 2018). As a result, both alone and in combination with *B. fragilis, B. cepacia* can improve the efficacy and tolerance of anti-tumor immunotherapy (Pitt et al., 2017). *Dysosmobacter welbionis* (Breton et al., 2022), *Eggerthellaceae* spp. (Lin et al., 2019), *Pediococcus pentosaceus* (Syakila et al., 2019), and *Butyricoccus pullicaecorum* are all probable NGPs (Andrade et al., 2020).

Prospects of next generation probiotics as biotherapeutics

Probiotics have been used as fermented foods by humans for a long time, but the beneficial effects of these bacteria were not well understood until recently. Probiotics of the current generation have already demonstrated their value in maintaining gut microbiota and reducing inflammatory responses, allergic illnesses, and autoimmune diseases (Tan et al., 2019). However, the restricted range of current-generation probiotics, as well as their limited survivability in meals and in the gut, makes it easier to find even better probiotics with greater beneficial qualities. Next-generation probiotics are the name given to such probiotics (NGPs). New microorganisms with potential beneficial characteristics to human health expand the probiotic spectrum and contribute to the development and elaboration of new food products that respond to the population's growing interest in health and quality of life, making next-generation probiotics an important topic for science and the food industry.

Despite various restrictions, the development of NGPs is moving forward. Isolation of such NGPs necessitates specialized culture techniques, and many such potential probiotics may persist in the gut environment in a viable but non-culturable (VBNC) form. Furthermore, in order to be employed as biotherapeutics, such NGPs must go through three rounds of clinical trials (preclinical, toxicological studies, and pharmacodynamics). Isolation of selected NGPs is predicted to be possible using advanced culturomics technology (Bilen et al., 2018). Furthermore, the availability of microbe-free animal models will aid in the identification of safe NGPs that meet regulatory parameters such as the US-LBP FDA's program (live biotherapeutic products) (Food and Drug Administration).

Although there is a strong interest among scientists to broaden the range of probiotic microbes, it poses significant obstacles for research and industry. The efficacy and safety of the next generation of probiotics, as well as the technological elements of using these microbes in food preparation, are the most pressing concerns. The great majority of these microorganisms have metabolic properties that make them challenging to utilize as sustainable methods in the development of novel products, particularly in large-scale food production (O'toole et al., 2017; Saarela, 2019).

The properties of these microorganisms, such as their great sensitivity to oxygen and remarkable adaptation to the gut habitat, make it difficult to prepare effective supplements. These bacteria, on the other hand, might be regarded as the next generation of probiotics. The discovery of physiologically active portions of these microbes will open up new avenues for health promotion research (Heintz-Buschart et al., 2018). The utilization of biotechnology techniques and information at various levels of '-omics' sciences (genomics, metabolomics) are valuable allies for improving probiotic strains and developing novel strains (Douillard and de Vos, 2019).

However, few studies have been conducted to establish the effectiveness and safety of these microorganisms, necessitating more research in *in vivo* systems and clinical trials. Finally, there are a few studies analyzing the potential application of next-generation probiotics in food matrices, and their impacts on intrinsic technical and sensory factors must be assessed. In the future, these microbes may be employed as biotherapeutic items and sold mostly as nutritional supplements.

Conclusion

Modern views about the role of the microbiota and microbiome in the formation and maintenance of health have sparked the development of several other next-generation probiotics, the consumption of which, in most cases, has a positive impact on the body owing to the microbiota modeling effect. Traditional probiotics, which are mostly *lactobacilli* and *bifidobacteria*, are increasingly giving way to next-generation probiotics from other families (most often deep anaerobes). In the future, more study into the mechanisms of action of these NGPs will allow probiotics to be used as biotherapeutics in the treatment of many disorders in both adults and children.

Author Contribution

MF conceived the idea and prepared the outline of the review. MF, MAS, ZY, and SSK performed the literature search and data extraction. MF, SSK and MAS analyzed the extracted data and wrote the manuscript. All authors read and accepted the final version of the manuscript.

Acknowledgment

Authors acknowledge their respective institutions and colleagues.

Competing financial interests

Authors have declared that no competing interest exists.

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