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Review Article

Integration of AI with Next - Generation Probiotics for Gut and Potential Health Benefits

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Abstract

The emerging discipline of microbiome research has significantly transformed our understanding of human health, particularly highlighting the potential of probiotics and fecal microbiota transplantation (FMT) as therapeutic interventions [1]. Although traditional probiotics have obvious benefits, their efficacy and mechanisms are yet unknown, and the long-term effects of FMT are still under investigation [2]. Advances in high-efficiency sequencing have gradually revealed gut microorganisms with obvious health benefits [3].

All of this is opening the path for next-generation probiotics (NGPs), which are designed via synthetic biology and bioinformatics to solve specific disease problems with greater stability and survival [4].

This review exposes our present knowledge on NGP stiffness, difficulties in administration, and their applications in avoiding and managing chronic illnesses like diabetes, obesity, and cardiovascular diseases [5]. We discuss the opportunities and challenges of including numerous NGP strains into therapeutic practice as well as explore their physiological aspects, safety profiles, and modalities of action, insisting on the need for further studies to guarantee their safety and improve their effectiveness [6]. NGPs have enormous potential to transform treatments/therapies and improve clinical outcomes that are microbiome-based.

Keywords: Synthetic Biology; Microbe Based Therapies; Next-Generation Probiotics; Chronic Diseases

Next generation probiotics features

Next-generation probiotics (NGPs) represent a novel class of beneficial microorganisms that are currently under intensive research and development for both therapeutic and nutritional applications [7].

Designed for use in medical interventions as well as in functional foods and dietary supplements, NGPs exhibit therapeutic potential for a range of chronic diseases, including metabolic disorders, cancer, and inflammatory conditions. NGPs possess unique attributes such as compatibility with synthetic biology,

gene editing, and personalized delivery systems, offering several advantages over conventional probiotics [8]. This review explores key research paths and the therapeutic implications of NGPs, with a focus on their impact on human health, including gut integrity and immune modulation.

Recent advances in next-generation sequencing (NGS), bioinformatics, and microbiological techniques like 16S rRNA PCR have enabled the precise identification and classification of novel intestinal bacterial strains [9]. Whole-genome sequencing and improved culturing techniques have further facilitated the isolation and characterization of new microbial candidates with significant probiotic potential [10]. These microbes—defined as "living organisms that, when administered in adequate amounts, confer health benefits on the host"—have expanded the traditional definition of probiotics to include species beyond *lactic acid bacteria* and *Bifidobacteria* [11]. Among the most promising NGPs are *Roseburia intestinalis*, *Eubacterium spp., Akkermansia muciniphila, Faecalibacterium prausnitzii,* and *Bacteroides spp.,* which are currently regulated under drug frameworks due to their novel status and therapeutic relevance.

Unlike conventional probiotics, NGPs offer advanced physiological benefits such as the production of folate, serotonin, indoles, and short-chain fatty acids (SCFAs), all crucial for host phenotype modulation [12]. However, the industrial development of NGPs is challenged by their requirement for enriched, often anaerobic culture conditions and expensive fermentation media [13]. Continued research is thus essential to optimize fermentation strategies and scale-up processes for commercial viability [11].

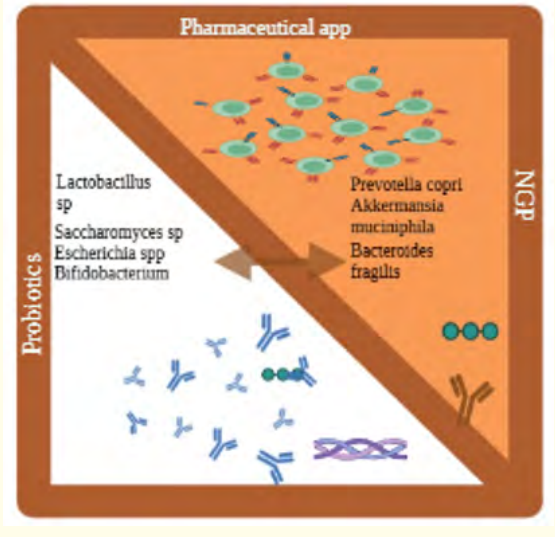

Figure 1: Transformation of Conventional Probiotics.

Different sources of microbial strains for next generation probiotics

Humans have been acknowledged as "Superorganisms" and are closely associated to microbiomes. Extensive study on Human microbiome is conducted that depicts that the human gastrointestinal tract (GIT) has trillion of bacteria, fungi, viruses, and protozoa**.** Expanding every bacterium can be a tough task as there is, lack of foregoing knowledge about the nutritional needs and appropriate growth conditions, of the dense bacterial inhabitants residing in the gastrointestinal system. Thus enabling researchers to determine/identify and quantify the many components of the gut flora, through the usage of bioinformatics tools and nucleic acid sequencing techniques has let them overcome this shortfall [14].

The diversity of gut flora is somewhat different in infancy and contains notable numbers of *Veillonella, Clostridium botulinum, C. coccoides*, *Bacteroides,* and *Akkermansia muciniphila* [15].

Following studies found that the first cholesterol lowering bacterium in a study by is the isolated *Bacteroides* sp. from human faces.

Correspondingly *Bacteroides fragilis* was filtered from a newborn that was fed from a healthy breast. This strain was able to treat immunological imbalance, inflammatory disease, mental disorders, polarise M1 macrophages and boost phagocytosis.

Another study found that the bacterial strain *Lactococcus lactis* may help alleviate Parkinsonism by reducing ferroptosis and correcting dysbiosis and oxidative stress in human cells [16].

S. no	Strain	Host	References
1	*Bacteroides fragilis*	Human feces	[27]
2	*Bacteroides dorei*	Human feces	[24]
3	*Lactococcus lactis*	GMO(Food)	[24]
4	*Bacteroides ovatus*	Human feces	[28]
6	*Clostridium butyricm*	Human	[29]
9	*Bacteroides fragilis*	Human feces	[30]
11	*Bacteroides fragilis*	Human Feces	[31]
12	*Bacteroides uniformis*	Human feces	[32]
13	*Akkermansia muciniphila*	Human Gut	[23]
14	*Roseburia intestinalis*	Human Gut	[23]
15	*Faecalibacterium prausnitzii*	Human gut	[33]

16	*Lactococcus lactis*	Fecal	[26]
18	*Lactobacillus rhamnosus*	Human blood	[34]
19	*Bifidobacterium lactis*	Human Blood	[34]
20	*Akkermansia muciniphila*	Human Blood	[35]

Table 1: Strains of Next Generation Probiotics.

Akkermansia muciniphila

The gram-negative bacterium *Akkermansia muciniphila,* a member of the *Akkermansiaceae* family [17], is among the finest prospects for NGPs. Although retracted in larger quantities (1-5%) under certain conditions, in healthy individuals this microorganism exists symbiotically and is a fundamental component of intestinal health. It resides primarily in the outer mucin layer of the intestinal epithelium and exclusively degrades mucin. This degradation process stimulates the production of new mucin proteins, thickens the mucus layer, and strengthens the gut barrier, thereby contributing to a balanced gut environment. Mucin itself, a glycoprotein naturally present in the human colon, acts as a "prebiotic" by nourishing mucin-degrading bacteria [17]. *A. muciniphila* is very vital for mucin metabolism since it strengthens the intestinal barrier and keeps harmful bacteria out of the circulation by stimulating mucus formation. Further support for immune regulation includes the upregulation of $Foxp3^+$ cells—a transcription factor essential for the development, function, and stability of regulatory T cells—as well as increased production of anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β) both reduces inflammation especially in conditions like Type 1 Diabetes (TID), where it helps lower islet inflammation.

People with a variety of metabolic disorders, including obesity, type 2 diabetes (T2D), inflammatory bowel disease (IBD), hypertension (high blood pressure), coupled with liver ailments, have reported lower abundance of *A. muciniphila*. Research on obese mice indicated that *A. muciniphila* can help reverse obesity brought on by a high-fat diet. It lowers metabolic endotoxins, promotes the release of lipopolysaccharides—which reduce inflammation—derived from bacteria, and increases insulin sensitivity. This suggests that restoration of *A. muciniphila* levels could help to reduce obesity and associated metabolic disorders [18]. *Akkermansia muciniphila* has been successfully pasteurized (inactivated), and studies on it demonstrated that heating it at 70 °C for 30 minutes significantly enhances its health benefits. The inactivated form of *A. muciniphila* has been shown to increase colonic length and crypt depth, improve obesity and insulin resistance, reduce body fat accumulation, and strengthen gut barrier function—exhibiting effects that are comparable to, or even greater than, those of the live bacterium.

Faecalibacterium prausnitzii

One very exciting next-generation probiotic is *faecalibacterium prausnitzii*. *F. prausnitzii* is taxonomically a member of the Ruminococcaceae family, phylum Firmicutes and class Clostridia. These are among the most ample microbes in individuals digestive tract. Recent research on the great genomic variation among *F. prausnitzii* strains revealed eight kind of groupings unique at the species level. Using average nucleotide identity (ANI) analysis, three newly identified species—*Faecalibacterium duncaniae, F. hattorii, and F. longum*—originated from the human gut were detected.

Found in the gut of healthy people, it makes roughly 5% of the total faecal microbiome**.** Actuall, insufficient amounts of it in the gastrointestinal tract have been linked to dysbiosis of bacteria, other metabolic disorders as well as chronic immune-mediated problems including obesity and inflammatory conditions. Usually utilized as a great biomarker for ulcerative colitis diagnosis in obese individuals, this can be used to heal injured intestinal mucosa and also promote the manufacture of mucin and tight junction proteins. *Faecalibacterium prausnitzii is* considered as a probiotic and plays a key role in protecting the human gut. When it is absent, the intestine loses important anti-inflammatory and immune-regulating functions. Short-chain fatty acids including formic acid, butyrate, a very low quantity of D-lactic acid, and salicylic acid Using *F. prausnitzii*, determine the most important products from glucose fermentation.

The health benefits of *Faecalibacterium prausnitzii* are attributed to its immunomodulatory effects, enhancement of intestinal barrier integrity, and anti-inflammatory properties. These functions help maintain intestinal homeostasis. The gut microbiota also plays a crucial role in intestinal and overall physiological processes by producing butyrate, a key short-chain fatty acid.

Actually, butyrate is the main energy source for the intestinal epithelial cells; it modulates the path of intestinal T-cells, limits the invasion of pathogens, facilitates death of colon cancer cells, reduces intestinal inflammation, regulates the body's defense, and aids in recovery from metabolic syndrome.

Given the above described characteristics, researchers have looked at individuals of the genus *Faecalibacterium* as regulators in cancer immunotherapy. For these reasons, *F. prausnitzii* is considered as a primary therapeutic target and prognostic indicator in melanoma patients.

By now *F. prausnitzii* isolated from healthy individuals has shown value for in-vitro anti-inflammatory and immunomodulatory effects. Although a research on calves has proven its safety, its prospective function and safety in humans remain unclear.

Bacteroides fragillis

Bacteroides fragilis is a long, rod-shaped, anaerobic, gram-negative bacterium that belongs to the *Bacteroides* genus. It is commonly found in the human gut as a commensal organism. Mother might pass it on to her child during delivery. As a result, it becomes the primary resident of the human gut and makes around 1% of the gut flora. Still, its presence in the female vaginal system, mouth, and upper respiratory tract is clearly apparent. Thus, *bacteroides* are opportunistic pathogens causing bacterial infections, IBD, human tumour immunity [19]. Through stimulation of adaptive immunity, reduction of inflammation, encouragement of immune system growth, and preservation of a healthy gut flora, these strains can strengthen the immunity. *B. fragilis* is mostly responsible for maintaining gut health and general stability by means of Polysaccharide A (PsA). Interestingly, treatment with this probiotic significantly improved gut bacterial diversity and increased the abundance of *Akkermansia muciniphila*. Reports also indicate that Bacteroides fragilis helps protect against Clostridioides difficile infection by preventing the loss of tight junction protein zonula occludens-1 (ZO-1) and mucus protein mucin-2 (MUC-2)—both of which are critical for gut barrier integrity. This protective effect occurs by inhibiting cell death, thereby preserving the intestinal lining.

Recent research has shown that *Bacteroides fragilis* breaks down various carbohydrates in the colon and produces unique capsular polysaccharides, including polysaccharide A (PSA)—a distinct amphoteric molecule known for its immunomodulatory effects [45]. Antigen-presenting cells process PSA and present it to T cells, thereby enhancing host immunity. PSA also influences dendritic cells, promoting the differentiation of T cells into regulatory T cells (Tregs). Furthermore, PSA reduces the production of the pro-inflammatory cytokine IL-17 and increases IL-10 secretion, helping to control intestinal inflammatory conditions such as inflammatory bowel disease (IBD).

Given these effects, treatment with either *B. fragilis* or purified PSA is considered a promising therapeutic strategy for individuals with autoimmune disorders, including IBD. Additionally, *B. fragilis* has shown benefits in cancer patients by enhancing immune function, reducing lipopolysaccharide (LPS)-mediated inflammatory signaling, and maintaining intestinal barrier integrity—thus preventing "leaky gut." In fact, a preclinical trial using *B. fragilis* as a live biotherapeutic agent reported it to be highly effective and safe, further supporting its potential as an innovative therapeutic tool.

Eubacterium halii

Another possible NGP for next probiotic biotherapeutic candidate is *E. halii*. Belining under the phylum Firmicutes, genus Eubacterium, *E. hallii* is a Gram-positive, uniform or pleomorphic, non-spore-forming, obligately oxygen-deprived rod-shaped bacterium. *Eubacterium* species are major gut bacteria that widely colonize the human digestive system across different populations, making up about 3% of the adult gut microbiota.

Eubacterium hallii is a chemoorganotrophic bacterium that can use various carbon sources like sugars and organic acids. It produces two key short-chain fatty acids, propionate and butyrate, which help maintain gut health by supporting epithelial cells, promoting the growth and development of intestinal cells (enterocytes), and increasing mucus production (Louis and Flint, 2017). The drop of SCFAs will cause an inflammatory reaction [20] thus the SCFAs, propionate and butyrate are known to be good for human health. First identified as a butyrate producer in the human gut, *E. hallii* was characterised by Barcenilla., *et al*. 2000. *Eubacterium hallii* is a key producer of butyrate in the gut,

especially in neonates [20]. Butyrate is formed in the colon from carbohydrates through bacterial fermentation, not in the stomach. Similarly, propionate is produced by gut bacteria during sugar fermentation. Studies highlight the importance of cross-feeding interactions between gut microbes in supporting short-chain fatty acid (SCFA) production by *Eubacterium* species. To investigate the potential role of *Eubacterium hallii* in metabolic disorders such as diabetes and obesity, researchers co-cultured *Eubacterium* spp. with *Bifidobacterium* spp. in media containing complex carbohydrates. *Bifidobacterium* strains have the enzymatic capacity to degrade complex sugars such as fucosyllactose and arabinoxylan oligosaccharides, which facilitates cross-feeding interactions. These breakdown products serve as substrates for *E. hallii*, supporting its growth and short-chain fatty acid production, thereby highlighting its potential therapeutic relevance in metabolic health.

Roseburia spp

Gram-positive, obligatory, anaerobic, curved rod-shaped *Roseburia spp*. are Gram-positive. It belongs to the family Lachnospiraceae, phylum Firmicutes, class Clostridia, order Clostridiales. In the stomach of a healthy adult person, the population of it is roughly 3-15%. Using intricate polysaccharides, this species generates SCFAs (propionate, butyrate, acetate). It is well considered as a potential NGP candidate and helps in inflammation, Parkinson's disease, inflammatory bowel disease (IBD). Remarkably, studies conducted lately, revealed that animals given Roseburia on a fiber-rich diet had lower atherosclerosis. Moreover, studies show how Roseburia intestinalis-derived flagellin, a key structural component of the bacterial flagellum, helps to manage alcoholic fatty liver and ulcerative colitis. For instance, in the alcoholic fatty liver mice model, oral intake of Roseburia intestinalis-derived flagellin greatly restored the integrity of intestinal epithelial cells and prevented the probable intestinal fistula ("A possible abnormal connection in the intestines). Similarly, [21] have found that flagellin produced *from Roseburia* intestinalis helps in colitis. The authors found that it had anti-inflammatory effects, which helped reduce intestinal inflammation and death in a mouse model of colitis. Still, the clinical data on *Roseburia* is somewhat lacking. More research is required to support the use of *Roseburia* in different human diseases.

Features	Conventional Probiotics	NGPs
Discovery and Development	Found through personal experiences and historical utilization of fermented foods	Discovered through cutting edge techniques such as bioinformatics and next-generation sequencing (NGS) studies
Safety	Generally considered as adequate for human consumption with extensive historical usage	Safety must be carefully evaluated due to new stress factors and engineered modifications.
Mechanism of Action	Mostly empirical, particular method of action not entirely known.	Explicit focused systems of action derived from metabolic and genetic engineering.
Application scope	Usually addresses broad, general issues of suboptimal health and digestive support.	Utilised to target particular diseases and ailments such as diabetes, obesity, cardiovascular diseases, and irritable bowel disease.
Formulation and Delivery	Generally consumed as dietary supplements in capsules, powders, and fermented foods	Potential to be used as biotherapeutics, possibly requiring specialized delivery systems.
Efficacy	Meant to interact with particular metabolic pathways and circumstances.	Higher efficacy with targeted action, designed to interact with specific metabolic pathways and conditions
Regulation	They have Established rules with a background of safe application.	Consist of emerging regulatory frameworks, with rigorous testing required for approval
Cost and Development	Usually cheaper because of proven manufacturing methods.	Potentially higher initial costs due to advanced R&D, but ccan be decreased with scaled production and standardization
Personalization	Limited ability to personalize, often a one-size-fits-all approach	Great potential for personalization, depending on unique microbiome compositions and particular health requirements

Table 2: Comparison of Ancient and Next Generation Probiotics.

Mechanism of Action of Next Generation Probiotics

Among the numerous modifications that probiotics can induce on the host are changes in the gut flora, modulation of the defense system, and synthesis of bioactive molecules. The particular strain or formulation will define how the NGPs affect immunological activity, intestinal health, and other health effects.

The next part of the article lists some basic NGP mechanisms:

- Lactic acid and short-chain fatty acids (SCFAs) boost the synthesis of peptides with antibacterial action including lactobin A, curvacin A, enterocin and pediocin in gut flora. These peptides strengthen the immune system and are essential for the defense against pathogenic germs by eliminating dangerous bacteria and so preserving a normal gut flora.
- Apart from their basic function in the breakdown as well as absorption of lipids, bile acids also function as signalling molecules in other metabolic functions. Also Abnormalities in bile acid metabolism have been linked to several metabolic and inflammatory diseases; therefore, the ability of NGPs to alter this process holds therapeutic potential.
- Furthermore, they compete with harmful bacteria for resources and space in the gut, strengthening the intestinal epithelial barrier and promoting mucus production. This, in turn, supports a healthy gut microbiota and helps slow the spread of pathogenic microbes. Thus by controlling the inflammatory response and affecting the behaviour of immune cells (T-cells and B-cells), probiotics can thus also alter the immune system.
- Apart from these immune-regulating properties, the gut flora also promotes the host's well-being by defending against infections, aiding in the digestion of indigestible food components, and producing essential metabolites.
- Often found in human, the putative probiotic candidates are *faecalibacterium prausnitzii, Eubacterium hallii, and Akkermansia muciniphila* [22].
- *Akkermansia muciniphila* helps inhibit harmful pathogens by breaking down intestinal mucin. Recent studies suggest it may serve as a marker of a healthy metabolic profile.
- *Faecalibacterium prausnitzii* is the principal source of butyrate; it can influence the expression of proteins that stabilize tight junctions and thus increases the integrity of the intestinal barrier in patients with IBD. Furthermore, F. prausnitzii influences host metabolism by breaking down glycoproteins in the gastrointestinal mucosa, which contain glucose, maltose, and N-acetylglucosamine.
- It also supports nutrient exchange in the gut by producing lactate and acetate.
- *Propionibacterium* plays a crucial role in immunomodulation through the expression of dihydrolipoamide acetyltransferase, which promotes the proliferation of Th17 cells and has been linked to a reduced risk of necrotizing enterocolitis. Thus *F. prausnitzii*, *E. hallii* and *A. muciniphila* are standout contenders among the identified keystone species, and they hold great promise for making a significant contribution to combate diseases arising from the dysbiosis of inflammatory and metabolic origins.

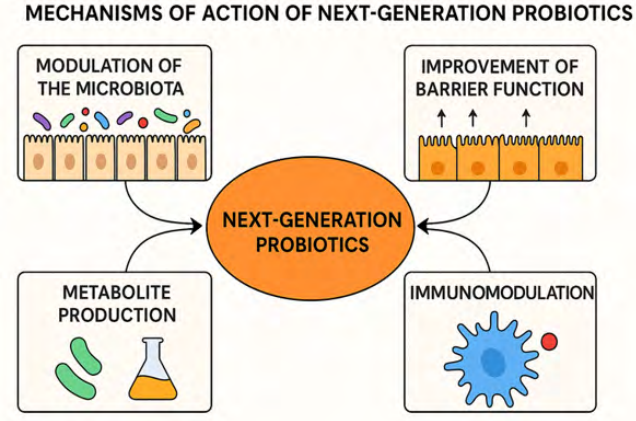

Figure 2: Mechanism of actions of next generation probiotics.

According to research, the primary ways to induce profitable alterations in the gut microbiome is by means of dietary alterations, achieved by minimizing processed, high-carbohydrate, or high-fat foods, as well as artificial sweeteners, sugar, and by broadening the intake of vegetables, fermented foods and fibers which promote the growth of beneficial bacteria.

Moreover, it is well established that the specific strain and dosage of probiotics determine their effects. Therefore, even within the same genus or species, the differing outcomes observed when using various probiotic strains can be partially explained. Additionally, factors such as the host's initial health status, the microbiota's capacity to accommodate new microbes, and the ecological niche shaped by the host's immune system during early life can all influence the outcomes of clinical trials involving probiotics—whether used alone or alongside other therapies [23].

Techniques for isolating NGP's and strategies to improve its stability and vitality

With the goal of developing more potent and specialized probiotic treatments, NGPs represent a potential new area in medical research. By altering the gut microbiota, increasing the effectiveness of conventional probiotics, and providing fresh treatment alternatives for chronic illnesses, these probiotics are intended to give certain health advantages. NGPs are intended to address particular health advantages by modifying the gut microbiota, in contrast to generic probiotics, which offer traditional benefits by promoting gut health only. NGPs are designed to improve the effectiveness of conventional probiotics by specific interaction with important microbial pathways, opening the door for novel therapies for a range of chronic illnesses, such as autoimmune diseases, metabolic disorders, and gastrointestinal disorders. To create these NGPs, a number of cutting-edge techniques are being investigated, such as gut microbiome engineering, CRISPR/Cas9 gene editing, and synthetic biology.

Synthetic biology

Through several complex interactions with systems such as the immunological, neurological, and digestive systems, the gut microbiota plays a crucial role in host metabolism. Synthetic biology is an interdisciplinary field that involves the design and construction of new biological parts, devices, and systems, or re-designing the existing biological systems, to perform specific functions. This includes engineering microorganisms, such as bacteria, to serve as probiotics with beneficial properties. By using this method, researchers can establish bacterial strains that are more resilient to harsh environmental conditions or that produce particular metabolites or proteins. For example, it is possible to design bacteria that create SCFAs, which can improve gut health and have anti-inflammatory qualities.

It is crucial to choose a suitable microbial strain for probiotic engineering since it needs to be adaptable, compatible with genetic tools, and able to colonize well. *E. Coli, Lactobacillus, Bifidobacterium*, and newer contenders including *Bacteroides, Clostridium butyricum,* and *Saccharomyces boulardii* are the most widely accessible bacteria. Each of them has unique benefits and difficulties.

The effectiveness of tailored probiotics relies on their ability to successfully colonize the gut. Techniques like surface modifications—using protective coatings such as silk fibroin or polysaccharides—can improve their survival and adherence in the gastrointestinal tract. A clear example of synthetic biology in this context is the genetic engineering of *Lactobacillus paracasei* to produce conjugated linoleic acid (CLA), a compound known to support weight loss in animal models. When this engineered strain was administered to obese mice, it significantly reduced body weight and fat mass while improving insulin sensitivity and glucose metabolism.

The ability of synthetic biology to produce probiotics with particular therapeutic roles was demonstrated when Peptide YY (PYY), an anorexigenic hormone that lowers appetite, was produced by engineering *E. coli.*

This strain was further administrated to obese rats, which resulted in decreased food intake, body weight, and fat mass [24].

CRISPR/Cas gene editing

Researchers can use CRISPR/Cas9 to add, remove, or change certain genes in a probiotic strain to make it last longer in the gut or make more helpful substances that could be used as medicines**.**

There are two types of CRISPR-Cas systems: Class 1 (multiprotein complexes) and Class 2 (single-protein effectors). Class 2 methods are often used in genome editing because they are easy to use.

Scientists have used CRISPR/Cas9 to get rid of genes that make Lactobacillus species sensitive to stomach acid. This helps the bacteria survive longer in the digestive system.

Cas9 has also been used to add genes that allow the production of anti-inflammatory chemicals to Bifidobacterium strains in order to make them better at treating diseases like IBD**.**

CRISPR/Cas9 was used to improve *Akkermansia muciniphila's* ability to make enzymes that break down mucin in a study involving this bacteria, which is known to be good for metabolic health. These enzymes make it easier for the bacteria to strengthen the gut barrier and reduce inflammation. This makes it better at treating metabolic diseases like diabetes and obesity. It was written by [25].

Incorporating CRISPR-Cas9 into probiotics enhances their stability and resilience to stress. Genes associated with stress reactions are overexpressed to achieve this. This enhances the synthesis of molecular chaperones and osmoprotectant mechanisms, hence improving the longevity and efficacy of probiotics within the host. CRISPR-Cas9 possesses significant potential applications in next-generation bacterial engineering; yet, it also presents several challenges. Many probiotic bacteria have little proficiency in homology-directed repair (HDR), complicating gene editing efforts. Nevertheless, contemporary techniques like Cas9 variations and base editors can circumvent these issues by facilitating precise modifications that preserve double-stranded DNA integrity**.**

GUT microbiome engineering

Gut microbiome engineering refers to alterations in the composition and function of the gut microbiota to promote health, prevent disease, or treat medical conditions. To get desired health results, this method can involve adding particular probiotic strains to the stomach or changing the present microbial population.

Fecal microbiota transplantation (FMT) a technique of gut microbiome enginnering is a method used to modify the gut microbiome by transferring stool from a healthy donor into the gut of a patient. It has been proven effective in treating *Clostridium difficile* infections (CDI) and is also being explored for conditions like metabolic syndrome and inflammatory bowel disease (IBD).

FMT works by restoring beneficial bacteria in the gut, which leads to high cure rates and low recurrence. In contrast, traditional antibiotic treatments for CDI can disrupt the gut microbiota and may contribute to antibiotic resistance. Although fecal microbiota transplantation (FMT) is not classified as a probiotic therapy, it highlights the potential of microbiome engineering to significantly alter gut conditions and improve host health.

Probiotics can be used to introduce helpful bacterial strains that are lacking in the gut microbiome of people with certain health conditions. For example, in patients with inflammatory bowel disease (IBD), *Faecalibacterium prausnitzii*—an anaerobic bacterium known for its anti-inflammatory effects—can help reduce inflammation and strengthen the gut barrier.

Similarly, people with gut-related disorders may benefit from *Bacteroides uniformis*, which supports gut barrier function and boosts immune responses [26].

Engineering the gut microbiome holds great promise for treating a variety of diseases and offers opportunities to develop innovative therapies.

Assessing the potential of next-generation probiotics for managing chronic diseases

Designed by sophisticated technologies like genomics and metagenomics [11]. NGPs are new and enhanced strains of helpful bacteria. Their possible health advantages could be even further enhanced by their engineering to generate certain molecules or chemicals with medicinal value [27].

Chronic diseases are long-lasting health problems that last for a year or more. They usually require ongoing medical care and can limit daily activities or both. NGPs are being developed to offer more-effective treatment for chronic diseases and solve limits of present probiotics. These new probiotics are meant to be more focused and offer more-specific therapeutic benefits for some ailments. Developing NGPs might be accomplished by engineering particular strains to express therapeutic chemicals or proteins. Lactobacillus bacteria can be modified to produce anti-inflammatory drugs like transforming growth factor (TGF)-β or interleukin (IL)-10.

These substances help reduce gut inflammation, which can relieve symptoms of inflammatory bowel disease (IBD). Therapeutic genes can also be delivered to the gut lining using *Lactobacillus* bacteria, offering potential treatments for conditions like ulcerative colitis (UC) and colon cancer [28].

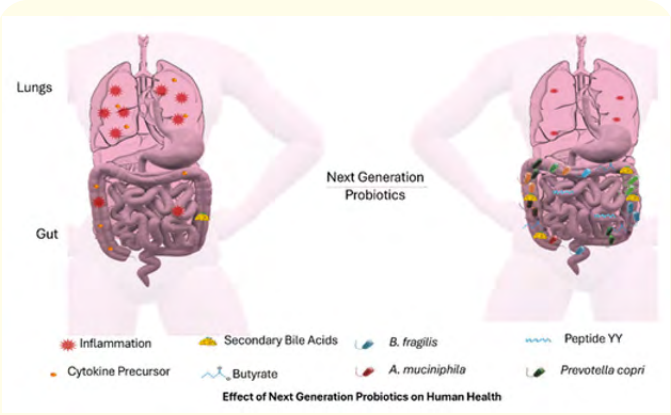

Figure 3: Role of NGP's On Human Health.

Additionally, specially engineered probiotics can be used to deliver specific drugs or vaccines directly to the gut, increasing their effectiveness while reducing side effects.

However, more research is needed to ensure their safety and effectiveness, and to develop cost-effective methods for manufacturing and distributing these next-generation probiotics (NGPs).

NGPs have the potential to treat a wide range of chronic diseases, including cardiovascular diseases (CVDs), diabetes, and IBD—conditions that require long-term care. Studies suggest that NGPs may help manage symptoms and improve outcomes in individuals with such chronic illnesses.

NGPs and cancer

Research on Next Generation Probiotics (NGPs) and their potential role in cancer treatment has focused on several cancer types, including colorectal cancer (CRC), gastric cancer, hepatocellular carcinoma (HCC), and cervical cancer**.** Stressors such as carcinogens and inflammation can disrupt the gut microbiome, increasing tumor susceptibility. Studies suggest that the mechanisms by which NGPs may influence colorectal cancer include promoting epithelial repair and barrier function, modulating inflammatory pathways involved in tumorigenesis, inducing apoptosis, upregulating beneficial cytokines, producing immunomodulatory metabolites (e.g., short-chain fatty acids, SCFAs), and selectively excluding pathogenic or tumor-causing microorganisms.

Ongoing investigations are exploring the antibacterial and pro-apoptotic effects of NGPs on human cancer cells. For instance, strains such as *Bifidobacterium animalis* subsp. *Lactis*, *Bacillus polyfermenticus*, *Lactobacillus acidophilus*, and *Bifidobacterium adolescentis* have been shown to inhibit the proliferation of colon cancer cell lines (CRC cell) [29].

Recent research has identified several NGPs that offer distinct health benefits, such as *Parabacteroides goldsteinii*, *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*, and *Bacteroides fragilis*. Among these, *A. muciniphila* holds particular promise for cancer immunotherapy, as it is enriched in the microbiota of patients who respond favourably to anti-PD1 treatment by increasing the abundance of cytotoxic T cells and regulatory T (Treg) cells. Additionally, *Butyricicoccus pullicaecorum*, which is rich in butyrate, has been shown to inhibit CRC cell proliferation and enhance the expression of tumor-suppressor genes such as SLC5A8 and GPR43, offering potential for cancer prevention and treatment [26].

The potential of next-generation probiotics (NGPs) in breast cancer treatment has been increasingly explored. For instance, *Lactobacillus plantarum* has demonstrated anti-cancer activity through downregulation of the NF-κB signaling pathway in *in vitro* studies using MDA-MB-231 breast cancer cells [30]. Additionally, a case study reported that regular consumption of *Lactobacillus casei* may be associated with a reduced risk of breast cancer among Japanese women, suggesting a potential chemopreventive effect with prolonged exposure. Furthermore, *Lactobacillus crispatus* has been shown to significantly inhibit growth, induce apoptosis, and suppress migration of the cervical precancerous cell line Ect1/E6E7 in a time-dependent manner via multiple mechanisms.

In addition, *Salmonella* has been explored for its cancer treatment potential due to its ability to release toxins, that selectively induce apoptosis in cancer cells. Furthermore, *Salmonella* can be used in cancer-specific immunotherapy by targeting tumor antigens and promoting immune system engagement with the tumor microenvironment. Although *Salmonella*-based cancer therapies are still in early clinical phases, they hold promise for combination therapies alongside conventional treatments such as chemotherapy, radiation, and surgery, particularly for advanced or multi-drug-resistant cancers [31].

In summary, the mechanisms through which NGPs may exert anti-cancer effects include the promotion of epithelial repair and barrier function, modulation of inflammatory pathways related to carcinogenesis, upregulation of cytokines, production of immunomodulatory metabolites, and the prevention of harmful biofilm formation. Despite the promising findings, further research is needed to comprehensively evaluate the therapeutic potential of NGPs in cancer treatment.

NGPs and neaurophysiological diseases

Probiotics were conventionally known for their contribution in sustaining gut wellness, but recent studies has shown that they can also have an intriguing application in treating various neurological and psychiatric disorders. Research and understanding of the gut-brain signalling methods involving the bacteria are still under progress. These could include modifications in the microbial setup, immune system activation, vagus nerve signalling, tryptophan metabolism, and generation of certain neuro-active chemicals by the bacteria. By means of the same mechanisms, the microorganisms also offer other neuroprotective advantages like reducing beta-amyloid fibril production and possessing antioxidant characteristics [32].

According to Tillmann., *et al*. probiotics influenced two important metabolic pathways in the body. They lowered the levels of dopamine and norepinephrine in the blood and increased the liver's production of S-adenosylmethionine (SAM) by supplying betaine, which reduced the use of methyl groups. Changes in these pathways are linked to various disorders, so probiotics might help treat them. In people with major depressive disorder (MDD), the probiotic strains *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 showed significant improvement in depression symptoms [33].

Low populations of *F. prausnitzii* have been linked to the severity of major depressive disorder (MDD) and bipolar depression. Additionally, it has been reported to possess anti-depressive and anxiolytic properties in chronically stressed mice.

A study conducted in Taiwan by Liu., *et al*. found that younger children with autism who took *L. plantarum* PS128 showed improved resistance and defensive behaviours. This suggests that this probiotic strain may help alleviate autistic symptoms like hyperactivity and disruptive behaviour [34].

Additionally, some studies have indicated that probiotics may reduce the risk of Alzheimer's disease by lowering inflammation and oxidative stress in the brain.

L. paracasei is another promising candidate for treating neurological diseases, including Alzheimer's, due to its neuroprotective effects through reducing inflammation and oxidative stress [35].

Probiotic *P. freudenreichii* P.UF1, derived from human breast milk, is also being studied for its potential benefits in supporting immune health and producing vitamin B12. Compared to other *P. freudenreichii* strains, *P. freudenreichii* produces significantly higher levels of vitamin B12, which is essential for proper brain function, the nervous system, and blood production.

Overall, NGPs show promise in treating a range of neurological and psychiatric conditions, including depression, anxiety, autism, ADHD, and Alzheimer's disease. They also have neuroprotective and antioxidant properties, helping to prevent beta-amyloid fibril formation.

NGPs and gastrointestinal disorders

NGPs (Next Generation Probiotics) are particularly effective in targeting gastrointestinal (GI) disorders and restoring microbial balance in the gut, which can improve digestive health and help prevent or treat various GI conditions key mechanisms of action include competing with harmful bacteria for nutrients and space in the gut, which helps reduce the growth of these bacteria, thus improving gut function and alleviating symptoms of many GI disorders. Additionally, the production of short-chain fatty acids and the improvement of gut permeability can help reduce symptoms of conditions like irritable bowel syndrome (IBS) and other gut-related issues [35].

Commonly used next-generation probiotic (NGP) strains include *Bifidobacterium pseudocatenulatum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and other *Lactobacillus* species, which have been shown to influence the serotonergic system in patients with irritable bowel syndrome (IBS) [35]. In a mouse model, *Faecalibacterium prausnitzii* was found to

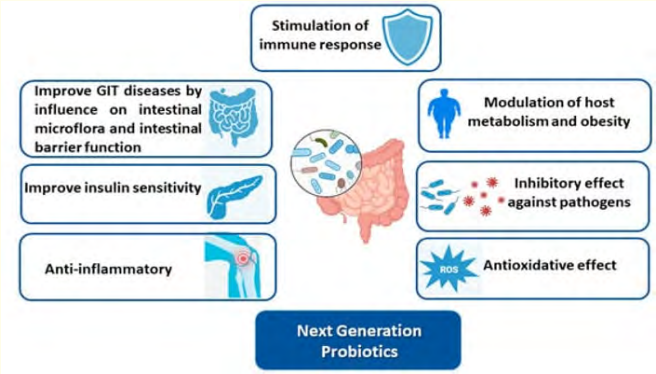

Figure 4: Health benefits of next-generation probiotics.

reduce intestinal permeability, lower colonic serotonin levels, and alleviate inflammation associated with low-grade inflammation and intestinal dysfunction. *B. bifidum* has also been shown to alleviate IBS symptoms like bloating and stomach discomfort, as well as reduce the risk of antibiotic-associated diarrhea. Additionally, fermented milk with *B. lactis* CNCM I-2494 helped improve symptoms in IBS patients with constipation [36] and *B. lactis* reduced gut hypersensitivity in stressed rats, protecting the colonic barrier from damage.

Probiotic strains such as *L. rhamnosus*, *L. plantarum*, and *L. reuteri* have been shown to reduce inflammation in the stomach, improve gut permeability, and lower the risk of diarrhea, thus alleviating symptoms of inflammatory bowel disease (IBD) [37].

In conclusion, NGPs hold significant potential for treating GI conditions such as IBS, IBD, ulcerative colitis, Crohn's disease, and diarrhea. They work by outcompeting harmful bacteria, boosting the immune system, regulating gut motility, producing short-chain fatty acids, and improving gut permeability. However, more research is needed to confirm the effectiveness of NGPs in treating these diseases.

Regulatory framework of Next generation probiotics

Probiotic regulations vary by country, and there is no single global framework. In the European Union, probiotics and food supplements are regulated under the Food Products Directive (2000/13/EU) and Regulation (178/2002/EC). The European Food Safety Authority (EFSA) must approve any health claims related to probiotics. Although EFSA has granted Qualified Presumption of Safety (QPS) status to several bacterial strains, it has so far rejected all submitted probiotic health claims. As a result, while product claims are strictly evaluated, the manufacturing process is not closely monitored, and post-marketing surveillance is minimal [38].

Government bodies such as the Food Safety and Standards Authority of India (FSSAI), the U.S. Food and Drug Administration (FDA) and the Dietary Supplement Health and Education Act (DSHEA) in the United States, Food for Specified Health Use (FOSHU) in Japan and the National Health Surveillance Agency in Brazil have gained international recognition to describe the regulatory pathways for probiotics marketed as medicinal products including clinical trial data or as components in functional foods [39].

One of the main regulatory challenges affecting the commercialization of next-generation probiotics (NGPs) is their classification, which can vary by region—being considered either pharmaceuticals or dietary supplements. This leads to different market pathways and compliance requirements. Inconsistent standards for safety and efficacy further complicate matters and increase the cost of meeting global regulatory expectations. Additionally, restrictions on health claims for probiotics make it harder for consumers to understand their benefits and complicate market positioning. Ensuring that all labeling meets regulatory requirements is crucial for maintaining consumer trust. These challenges highlight the need for harmonized regulations to support the global acceptance and commercial success of NGPs.

Safety and effectiveness considerations for Next generation probiotics

The concept of next-generation probiotics (NGPs) was formally introduced by *Nature Microbiology* in 2017. NGPs are considered to meet the criteria for "active biological agents" under U.S. FDA regulations and differ from traditional probiotics [40]. Before being marketed, NGPs must undergo regulatory approval and clinical trials [41]. Candidate NGPs are now expected to meet requirements related to safety, personalized therapeutic effects, and their interactions within the host microbiota [42]. However, safety concerns can vary significantly even among strains of the same species. Thus, safety remains the most critical factor in the development of NGPs. Different countries have distinct approaches

to evaluating NGP safety. In the European Union, the European Food Safety Authority (EFSA) is responsible for assessing the safety of new food products, including NGPs.

Accurate species-level classification, whole-genome sequencing, and assessment of antibiotic resistance (including the risk of horizontal gene transfer) are essential parts of evaluating microbial strains. It's also important to identify any harmful metabolic traits, such as those linked to obesity, diabetes, or metabolic syndrome [43]. Advanced methods like next-generation sequencing (NGS) and bioinformatics are useful for screening and isolating NGPs [44]. Human clinical trials and tests of genetic stability are also necessary to ensure the safety and effectiveness of NGPs. These steps help confirm their reliability and potential for various medical applications.

Important research on next-generation probiotics (NGPs) uses multiple approaches to assess their safety and efficacy. These include clinical studies such as randomized controlled trials and longitudinal studies, which employ validated outcome measures like symptom severity scores, biomarkers, and quality of life assessments. Before progressing to human trials, animal models are often used to explore mechanisms of action and establish preliminary safety profiles. Interventions typically involve the controlled administration of specific probiotic strains or formulations, while participant selection criteria usually consider factors such as age, health status, and the specific conditions being studied.

Challenges and opportunities of next-generation probiotics in clinical practice

The application of next-generation probiotics (NGPs) in clinical practice presents both challenges and opportunities (Table 3). One major challenge is the need for comprehensive research to establish their efficacy and safety. While early-phase clinical trials and preclinical studies provide promising results, more robust and targeted investigations are needed to confirm the effects of NGPs in treating specific chronic conditions. Another challenge is the complexity of the gut microbiome and the need to address individual variability in microbial composition and function. This highlights the importance of personalized approaches to maximize the therapeutic potential of NGPs [45].

Obstacles	Opportunities
Regulatory obstacles in launching new probiotics to market	Invention of tailored probiotics depending on personal microbiomes
Selecting the right strain or mix of strains for a certain ailment is challenging.	More knowledge of the probiotics and the microbiome mechanics
Limited understanding of the long-term effects of probiotic use	Potential for probiotics to be used as low-cost, low-risk treatment options
Restricted knowledge in probiotic products and dosages	Advances in technology, such as CRISPR/Cas9, for designing and engineering probiotics
Risk of unfavourable outcomes in certain populations, such as immunocompromised individuals	Possibility to lower linked hazards and antibiotic use, including resistance.

Table 3: Challenges and Opportunities of Next-Generation Probiotics.

One of the main challenges in using next-generation probiotics (NGPs) is ensuring their stability and survival in the gastrointestinal tract, where they face harsh conditions such as low pH and bile salts.

On the other hand, NGPs offer several promising opportunities. Their targeted and specific actions may enhance their effectiveness compared to conventional probiotics. Additionally, the ability to design probiotics with defined purposes and mechanisms opens up new therapeutic strategies for managing chronic diseases. NGPs may also cause fewer side effects than traditional treatments, such as medications, which could improve patient quality of life and treatment adherence. Finally, by reducing the need for expensive therapies and hospitalizations, NGPs could help lower healthcare costs.

Although the adoption of NGPs in clinical practice presents certain challenges, their potential benefits make this an exciting area of research and development.

The Applications of AI for microbiome research and probiotic medication administration

Artificial intelligence has evolved into potent measure for analysing and assessing the several and complex data on the microbiota [10] in recent years. Because of its ability to manage enormous volumes of data and identify hidden trends, artificial intelligence offers new opportunities to better understand the interactions between the microbiota of the gut and human health [47].

This paper aims to study how artificial intelligence can be used to detangle gut microbiome functions, influencing various disorders. Recent research employing artificial intelligence techniques to evaluate microbiome data, identify biomarkers, and project treatments responses [46,48]. Furthermore under debate are probiotic treatments aimed to alter the gut flora in order to improve host condition. Artificial intelligence can greatly help to maximise these interventions depending on individual's microbiota profiles and enable the most effective probiotic combinations.

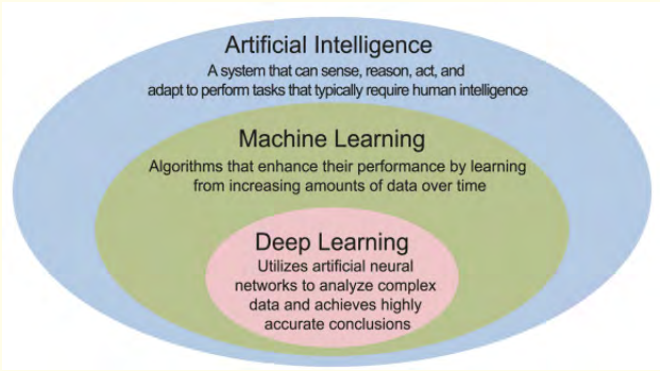

Figure 5: Hierarchical Relationship Between Artificial Intelligence, Machine Learning, and Deep Learning.

AI's effect on microbial analysis

AI has revolutionized microbiological diagnostics by offering unprecedented speed, accuracy, and analytical depth. Traditional methods for microbial identification and characterization often rely on complex molecular biology techniques or labor-intensive culture-based approaches. One of the most significant advancements is the use of machine learning algorithms to analyze high-throughput sequencing data for pathogen detection. For example, [48] developed a deep learning model called *DeepMicro*, which uses metagenomic sequencing data to accurately identify bacterial species with high sensitivity and specificity. This approach allows for the simultaneous detection of multiple pathogens, including those that are difficult to culture or present in low abundance. Additionally, AI is enhancing the interpretation of antimicrobial susceptibility testing results, further improving diagnostic precision and treatment decisions.

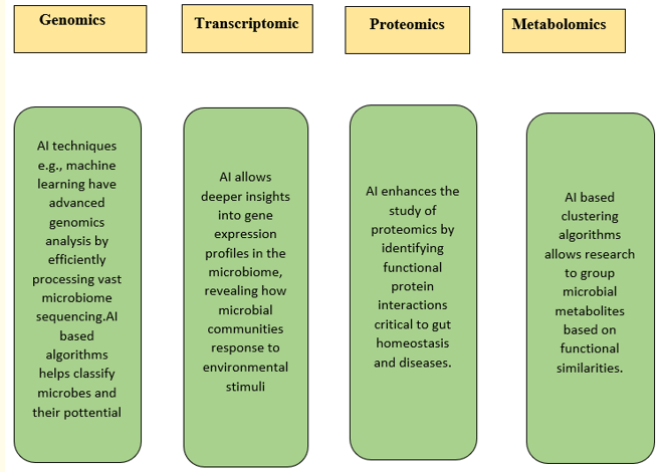

Figure 6: This graphic illustrates the integration of AI into many domains of microbiome investigation, depicting the progression from data analysis (Original).

Despite the encouraging developments, several challenges need to be solved for the use of artificial intelligence to microbiome research and antibiotic resistance. These include the following:

- **Standardization of data quality**: Developing effective artificial intelligence models requires consistent, high-quality data across various platforms and research projects**.**
- **Interpretability**: Many AI models, especially deep learning approaches, function as "black boxes", making their decision-making processes difficult to understand**.**
- **Integration of multi-omics data**: A major challenge remains in developing AI models that can effectively integrate diverse data types—such as metagenomics, metabolomics, and host genomic data—for comprehensive analysis**.**

- **Ethical issues**: The use of AI in healthcare raises significant ethical concerns, including algorithmic bias, data privacy, and the impact on clinical decision-making**.**

The final comments and Future Viewpoint

An major advancement in biomedicine is the inclusion of artificial intelligence into studies of the microbiome. Comprehensive data research and machine learning techniques have facilitated an unparalleled understanding of the intricate relationships between human health and gut microbiota. Superior resolution profiling of microbiome facilitates the identification of specific bacterial strains is absolutely essential for human physiological functioning and related illnesses.

Moreover, artificial intelligence allows thorough functional profile, pointing up metabolic deficits treatable with targeted probiotic therapies.

High-throughput sequencing catches the variety and intricate nature of microbial communities as massive amounts of microbiome data are generated. Strong artificial intelligence systems can to examine this data, identify trends, and create forecasts on health outcomes—such as recognising suspected disease indications or approximating the effectiveness of probiotic treatments. These technologies taken together show a considerably improved knowledge of the microbiome and how it affects human well being, as well as establishes the groundwork for the advancement of artificial intelligence (AI)-driven methodologies in microbiome-based therapies.

Configuring probiotic treatments is made possible by AI-driven algorithms projecting specific responses depending on host genetic and immune-mediated traits. This customised approach looks to reduce side effects, enhance therapeutic outcomes, and significantly boost the potency of probiotic therapy. Still, many problems must be addressed if we are to fully realise the possibilities of these technologies. First of all, the standardising of production, formulation, and quality oversight of probiotics determines the accuracy and dependability of the results. Moreover, it is necessary to better understand the mechanism by which probiotics generate their effects to generate more exact prospective treatments. Enhancing probiotic pharmaceuticals necessitates advanced algorithms and extensive databases capable of encapsulating the complexity of host-microbiota interactions. At last, ethical and legal aspects must be carefully investigated to ensure the safety, equity, and availability of the application of artificial intelligence in microbiome research. In spite of these challenges, the mix of artificial intelligence with other cutting-edge technologies looks to change our understanding of microbial populations opening the road for customised, microbiome-based treatments. By means of multidisciplinary cooperation and additional research, we can develop innovative therapeutic strategies improving human health and effectively addressing a wide spectrum of harmful disorders.

Conclusion

A revolutionary development in the field of microbiome-based treatments, next-generation probiotics (NGPs) offer a potential strategy for the prevention and treatment of chronic illnesses like diabetes, obesity, cardiovascular disease, and inflammatory bowel disease (IBD). In comparison to conventional probiotics, the current research highlights the possibility of these enhanced probiotics to offer therapeutic interventions that are safer, more effective, and more tailored. Researchers are creating probiotics that can modify the gut microbiota with previously unheard-of accuracy, improving therapeutic results, by utilizing cutting-edge technologies like synthetic biology, CRISPR/Cas9 gene editing, and gut microbiome engineering. The creation of NGPs overcomes a number of drawbacks of conventional probiotics, such as their ephemeral benefits, lack of consistency, and individual differences in effectiveness. The therapeutic potential of NGPs is increased by their increased resilience, targeting, and customization to specific microbiomic compositions. Examples of novel strategies being used to treat chronic illnesses include modified strains like Akkermansia muciniphila, which enhances glucose metabolism, and Lactobacillus paracasei, which produces conjugated linoleic acid (CLA). Notwithstanding the encouraging outcomes of preclinical research and early-stage clinical trials, the area of NGPs still confronts many obstacles, mostly related to regulatory approval and the requirement for substantial study to prove long-term safety and efficacy. Although promising, personalized approaches necessitate a more thorough comprehension of the intricate relationships that exist between probiotics and the host microbiota. To ensure that these cutting-edge probiotics fulfill strict safety and effectiveness requirements, regulatory frameworks must change to facilitate their commercialization and clinical application.

There are a lot of prospects related to NGPs. They have the potential to improve adherence among patients and quality of life by providing more targeted, efficient interventions with fewer adverse effects than conventional therapies. Additionally, by lowering the need for costly procedures and hospital stays, the adoption of NGPs may result in financial savings for healthcare systems. Continued study and development, with an emphasis on comprehending their mechanisms of action and maximizing their therapeutic potential, is essential for the future of NGPs. The scope of NGPs will be further expanded as novel microbial strains with possible health benefits are discovered thanks to advancements in bioinformatics and high-throughput sequencing. NGPs have the potential to be a key component of personalized medicine, providing focused treatments for chronic illnesses and enhancing general health outcomes with the backing of regulatory bodies and thorough clinical trials.

Lastly, NGPs have a lot of potential to improve chronic illness management and advance customized medicine. Unlocking the full potential of NGPs requires ongoing study and development, aided by changing regulatory frameworks and technical developments. NGPs are anticipated to become more crucial in promoting health and treating a variety of chronic illnesses as our knowledge of the gut microbiota grows, which would represent a major advancement in the field of probiotic medicine.

Author Contribution

Rajesh Kumar; Project Administration and Supervision, Saumya Tiwari; writing -original draft preparation, Suman Upadhyaya; conceptualization, supervision, Satyndra Kumar Yadav; review and editing, Neha Vishnoi; editing. All authors have read and agreed to the published version of the manuscript.

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Data Availability

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Competing Interests

The authors declared no competing interests.

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