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Review

Understanding the Functionality of Probiotics on the Edge of Artificial Intelligence (AI) Era

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Abstract: This review focuses on the potential utilization of artificial intelligence (AI) tools to deepen our understanding of probiotics, their mode of action, and technological characteristics such as survival. To that end, this review provides an overview of the current knowledge on probiotics as well as next-generation probiotics. AI-aided omics technologies, including genomics, transcriptomics, and proteomics, offer new insights into the genetic and functional properties of probiotics. Furthermore, AI can be used to elucidate key probiotic activities such as microbiota modulation, metabolite production, and immune system interactions to enable an improved understanding of their health impacts. Additionally, AI technologies facilitate precision in identifying probiotic health impacts, including their role in gut health, anticancer activity, and antiaging effects. Beyond health applications, AI can expand the technological use of probiotics, optimizing storage survival and broadening biotechnological approaches. In this context, this review addresses how AI-driven approaches can be facilitated by strengthening the evaluation of probiotic characteristics, explaining their mechanisms of action, and enhancing their technological applications. Moreover, the potential of AI to enhance the precision of probiotic health impact assessments and optimize industrial applications is highlighted, concluding with future perspectives on the transformative role of AI in probiotic research.

Keywords: omics technologies; biotechnological optimization; AI-driven probiotic research; precision health; microbiota modulation; metabolite production; viability

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1. Introduction

Probiotics, "live microorganisms which, when administered in sufficient numbers, impart a health benefit on the host" [1], have been under intense investigation for their contribution to the promotion of gastrointestinal wellness, immune system regulation, and disease prevention. Probiotic strains, which belong primarily to the genera *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, and *Bacillus*, achieve beneficial effects through various mechanisms that include modulation of microbiota, competitive exclusion of pathogens, production of antimicrobial substances, and enhancement of gut barrier function [2,3]. Besides, next-generation probiotics (NGPs) are recently identified as health-boosting microorganisms, typically of gut commensal origin, and discovered through advanced microbiome

analysis, with distinct health advantages over conventional probiotics [4]. Functionally, NGPs show their positive impacts by yielding bioactive metabolites, influencing host immune responses, and strengthening the intestinal barrier [5].

Maintaining the viability and functionality of probiotic strains under several physiological and storage conditions is one of the main difficulties in probiotic research. The probiotics should survive in the hostile gastrointestinal (GI) environment including gastric acid, bile salts, and digestive enzymes, to colonize themselves in the intestine [6]. In addition, maintaining probiotic viability in pharmaceutical products and food systems is crucial for their effectiveness, as temperature variation, oxygen exposure, and prolonged storage dramatically impact cell viability [7]. Novel technologies or approaches are required to enhance probiotic stability, optimize their bioactivity, and enable real-time monitoring of their viability throughout storage and manufacture.

Probiotic research has been facilitated by the new omics technologies that encompass transcriptomics, proteomics, metabolomics, and genomics, generating more information about the genetic and functional characteristics of probiotics. Genomics made it possible the sequence of the probiotic strains, which gives information about the genes towards adhesion, aggregation, viability, and metabolic processes [8]. Besides that, NGPs, such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*, have also been subjected to taxonomization effectively using comparative genomics [5]. Transcriptomics gives us description data regarding the expression patterns of genes under various states of the environment and enables us to know more about the reaction of probiotics towards stressors such as temperature, bile salts, oxidative stress, and acid stress [9]. On the other hand, the production of metabolically produced end products like peptides, bacteriocins, exopolysaccharides, and short-chain fatty acids (SCFAs), which play a crucial role in probiotic function and host-microbe interaction, has been investigated by proteomics and metabolomics [10,11].

The field of microbiology has greatly benefited from AI by paving new avenues for exploring the science behind probiotics. Algorithms like machine learning [12] and deep learning (DL) make it possible for multi-omics data integration, which increases the ability to predict probiotic behavior, adaptation, and even possible therapeutic applications [13]. AI systems powered by DL networks can estimate how probiotics will react with gut microbiota, predicting useful and harmful interactions to develop accurate analyses of the microbiome [14]. Additionally, AI can model how probiotics eliminate pathogens, providing insight into the production of antimicrobial peptides [15] and quorum-sensing mechanisms [16] by ML. On top of that, accelerated AI analysis of complex metabolic networks can be used to enhance the fermentation process [17,18]. Such advancements could pave the way for the creation of functional foods enriched with one or more ingredients to address specific health issues. With support from AI, the immunomodulatory properties of probiotics can be analyzed through the analysis of both host-microbe interactions and cytokine profiles [19,20].

Beyond gut health, probiotics have been studied in detail for their potential health benefits, including anti-cancer properties, anti-aging effects, and urogenital health benefits. AI could help with precision health by providing personalized probiotic products designed based on the composition of an individual's microbiome [21]. It has been reported that AI models can predict the success of probiotics in the treatment of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) [12,22]. The identification of anticancer and anti-inflammatory probiotic metabolites, such as SCFAs, that are capable of modifying the gut microbiome were discovered using AI molecular docking studies [23]. Moreover, the use of AI advances probiotic studies on skin and osteoporosis problems [24,25].

The minimum number of viable probiotics in food products throughout production, storage, and shelf life poses a challenge to efficacy. AI tools such as computer vision and

biosensors have been suggested to monitor probiotic viability in real time [26]. On the other hand, AI algorithms have been developed to monitor temperature, pH, and humidity and predict their impact on probiotic survival, leading to enhanced stable formulations [27,28]. Beyond survival, stability, and shelf-life optimization, AI can further increase probiotic efficacy through additional biotechnological means. For this purpose, synthetic biology powered by AI can be used to trigger silent genes responsible for probiotic bioactivity like stress resistance and bacteriocin synthesis [21,29–31]. AI techniques can also be utilized to enhance the sporulation efficiency of *Bacillus*-based probiotics and their tolerance to extreme processing and storage conditions [32].

Even though there is a growing interest in the role of AI in analyzing probiotics, the majority of the existing studies have focused on topics such as health promotion, therapeutic applications, gut microbiota interactions, gastrointestinal system prediction, personalized healthcare, or industrial process optimization separately. To the best of our knowledge, so far, no study has attempted to provide the impact of AI on probiotic science with a comprehensive systemic approach that combines all these distinct areas. In this regard, the current review integrates diverse lines of research to construct a multidimensional framework, emphasizing the substantial role of AI in the advancement of probiotic science. This review presents a comprehensive view of the strategic implementation of AI technologies in advancing various aspects of probiotics, including characterization, functional enhancement, application in health, and innovation in technology. It also describes the implications of AI technologies for improving strain selection, optimization of the production process, enhancement of probiotic stability and viability, and probiotic development, thereby offering new perspectives for scientific investigations and industrial applications.

2. Current Knowledge of Probiotics

Thanks to the undeniable benefits of probiotic microorganisms, the concept of probiotic foods is gaining significant interest among consumers, driving the search for new isolates with commercial potential [33,34]. Therefore, many researchers are actively working to discover new isolates from several sources [35–37], conducting a wide range of in vitro analyses in the initial stages. These analyses are evaluated under two main headings: functional and technological characteristics. Functional properties, in particular, are classified into four key categories: survival during delivery to the target organ, interaction with host systems, antipathogenic effects, and safety [38,39].

The capacity of strains to remain viable and active in adverse gastrointestinal tract (GIT) environments, as well as throughout the processing and storage of probiotic products, is one of the main requirements for consumers to obtain the claimed benefits of probiotics [40].

One of the crucial cell surface characteristics that facilitates the adhesion and colonization of probiotics within the human GIT is the auto-aggregation property [41]. Also, this feature is effective for a variety of microbial traits, including biofilm formation, colonization, and pathogenicity [42]. Co-aggregation of probiotic microorganisms is also an important index to combat and resist the intestinal invasion of pathogens, and this significantly contributes to the establishment of a defense mechanism, particularly against infections [43,44].

Another crucial characteristic of probiotics is their hydrophobicity, which influences the aggregation and adhesion capabilities of microorganisms [45]. The lack of toxic metabolite synthesis [46], virulence, and antibiotic resistance of the microorganisms [47] are critical parameters that indicate the safety of probiotics. For example, the possible health risk associated with the transfer of antibiotic resistance genes from probiotics to bacteria within the resident microbiota of the human GIT, and consequently to pathogenic bacteria, is a cause for concern [48].

Functionally recognized potential probiotics are also expected to exhibit antimicrobial effects through various mechanisms including competition, exclusion, and displacement,

which is also recognized as another important criterion for probiotic action [49,50]. Bacteriocins, hydrogen peroxide, and various organic acids are key components produced by potential probiotic microorganisms, possessing antimicrobial activity and leading to the aforementioned mechanisms [51]. These microorganisms recognized as probiotics because of their beneficial properties, contribute significantly to health when consumed, not only due to their presence but also through the bioactive metabolites they generate [52]. Among these secreted secondary metabolites are different organic acids, SFCAs, enzymes, peptides, peptidoglycans, exopolysaccharides (EPSs), vitamins, neurotransmitters, and amino acids [53–55]. In terms of health effects, these secreted compounds exhibit various biological activities, including antiviral [56], antifungal [57], anticancer [58], anticholesterol [59] properties, and neurological effects [60], etc., as seen in Table 1. All of these mentioned features are strain-specific [61] and have low feasibility of being realized on hundreds of isolates within the scope of research, leading researchers to search for alternatives.

Table 1. Recent studies about biological activities of metabolites secreted by probiotics.

Metabolite	Type	Microorganism	Biological Activity/ Key Outcomes	Reference
Exopolysaccharides	α-glucan	*Pediococcus acidilactici* NCDC 252	Anticancer (human colon cancer cell line)	[62]
	Heteropolysaccharide	*Lactiplantibacillus paraplantarum* NCCP 962	Cholesterol-lowering	[59]
	Heteropolysaccharide	*Limosilactobacillus fermentum* NCDC400	Cholesterol-lowering	[63]
	-	*Lactiplantibacillus plantarum* MI01	Anticholesterol	[64]
	-	*Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 20081	Antioxidant, antitumor, periodontal regeneration	[65]
	Negatively charged acidic Galactoglucan and levan	*Lactiplantibacillus plantarum* SN35N	Antiviral	[56]
		Lactococcus lactis F-mou	Antimicrobial	[57]
	Glucomannan	*Lactiplantibacillus plantarum* BR2	Antidiabetic, cholesterol-lowering, and antioxidant	[66]
Vitamin	Vitamin B	*Bifidobacteria* spp.	Secretion pyridoxine (B6): 0.988–26.060 mg/L	[67]
		Lactic acid bacteria spp.	Secretion pyridoxine (B6): 1.100–11.400 mg/L Secretion pantothenic acid (B3): 3.966–138.600 mg/L Secretion thiamine (B1): 14.720–19.540 mg/L	
		Lactiplantibacillus plantarum HY7715	Secretion riboflavin (B2): 34.5 ± 2.41 mg/L	[68]
	Vitamin B_2 and B_9	*Leuconostoc mesenteroides* subsp. *mesenteroides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*	About 1.7–32-fold increase in quinoa sourdough	[69]
	Riboflavin (B_2)	*Lactiplantibacillus plantarum* M5MA1-B2	About 2.5-fold increase in oat kefir	[70]
Short-chain fatty acids	Butyrate	*Lacticaseibacillus paracasei* SD1 and *Lacticaseibacillus rhamnosus* SD11 *Clostridium butyricum*	Anticancer and anti-inflammation	[71]

Table 1. *Cont.*

Metabolite	Type	Microorganism	Biological Activity/ Key Outcomes	Reference
Postbiotic metabolites	Organic acids, acetoin, 2,3- butanediol	*Leuconostoc pseudomesenteroides* Y6	Anticancer	[72]
	-	*Lactobacillus plantarum*	Anticancer	[73]
	Organic acids	*Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactobacillus gasseri*	Antifungal	[74]
	Organic acids, volatile organic compounds, polyphenols	*Lacticaseibacillus rhamnosus*	Antiaflatoxigenic	[74]
	3-phenyl-1,2,4-benzotriazine	*Lactiplantibacillus plantarum*	Anticancer	[75]

In addition to probiotics belonging to genera such as *Bacillus*, *Lactobacillus*, and *Bifidobacterium*, which are responsible for fermentation and whose consumption is reported to have positive effects on human health, the human GIT has also been identified as an alternative isolation source for microorganisms with probiotic potential [76]. In this context, new terms have emerged regarding probiotics, such as NGPs, Live Bio-Therapeutic Products, prebiotics, symbiotics, postbiotics, and paraprobiotics [77]. The discovery of species that had not previously been used in the food industry has led to the development of isolates referred to as NGPs. These microorganisms have a significant impact on human health with the metabolites they produce. Consequently, the discovery of new NGPs has become increasingly important for the prevention of critical diseases [78]. However, critical stages such as determining the species that meet the required safety criteria, especially with whole genome analysis, and ensuring their suitability for consumption by different consumer groups, pose limitations to the use of NGPs. Another critical factor is that NGP production is somewhat more complex than other alternatives [79]. Studies should also be supported by detailed in vivo analyses and human trials as well as AI tools. In addition to the health effects that should be taken into account, appropriate experimental plans and designs should be carried out to examine difficult-to-analyze parameters such as the dose, duration, and amount of probiotics to be consumed [80]. Consequently, ongoing research is crucial to fully understand their mechanisms and to ensure their safety and efficacy for future therapeutic uses and AI tools could be a powerful perception to advance probiotic research.

3. Exploring Probiotic Characteristics via AI

3.1. Omics Technologies

Multi-layered analyses targeting different molecules enable a more comprehensive insight into probiotic characteristics or their effect on human health, beyond traditional phenotypic approaches. Omics technologies, which form the basis of these analyses, have reached a very important point for the production and evaluation of multidimensional data in modern biological science. Omics is defined as an approach that enables the analysis of large-scale data on the structure and function of biological systems, enabling the holistic and effective examination of these systems through the integration of top–down and bottom–up strategies [81]. Omics is a comprehensive study in which molecules such as DNA, RNA, proteins, lipids, and metabolites are examined in detail to identify differences and reveal a mechanism or features [82]. Accordingly, omics technologies include genomics, transcriptomics, metagenomics, proteomics, lipidomics, and metabolomics. However, to understand biological systems in a holistic and more comprehensive way, the multi-omics

approach has recently come to the fore. This approach implies the combined use of different omics. In particular, it has been recommended to use different omics technologies together to achieve a holistic approach to determining the properties of probiotics, understanding their interaction with the host, and their selection [13].

With genomic, transcriptomic, proteomic, lipidomic, metabolomic, and metagenomics approaches, both the characteristics of probiotic strains and their biological responses to environmental conditions can be revealed in detail. In multi-omics approaches, datasets are processed with various statistical methods [83]. However, the datasets generated by these technologies exceed classical analysis techniques in terms of volume, diversity, and complexity, causing difficulties in data integration [84]. This has highlighted AI-based methods as a powerful tool for analyzing and integrating omics data to unravel the complexity of datasets [85]. By integrating this multi-layered biological data, AI algorithms can uncover previously undiscovered biological patterns, accelerate probiotic selection, and optimize product development processes [86].

Genomics, which is the basic step of the omic analysis, maps the genetics of probiotic microorganisms by presenting their DNA sequences. With the whole genome sequencing of a probiotic candidate, information about thousands of genetic elements can be obtained [87]. The spaCBA pilus gene cluster, which contributes to mucosal adhesion capacity, was identified in the *Lacticaseibacillus rhamnosus* GG [88]. In another study, functional genes such as replication, recombination, repair, or defense mechanisms that facilitate adaptation to the intestinal environment were identified in the probiotic *Lactobacillus johnsonii* ZLJ010 [8]. The existence of these genes can be used to predict functional properties in AI-supported classification systems. Data obtained through genome analysis mainly consist of DNA base sequences, and the identification and processing of meaningful information on DNA is expressed as annotation. In genomic analyses, secondary information such as gene/protein functions, mobile genetic elements, and antibiotic-resistance genes is also extracted during the annotation phase [89]. Such data provide multidimensional labeled datasets for AI systems.

Genetic information alone may not be sufficient to explain the response of microorganisms to environmental conditions. For this reason, transcriptomic analyses allow us to determine at what level genes are active under which conditions [9]. Data obtained with RNA-seq technologies include the transcript amount of each gene (with units such as FPKM and DEGs) and alternative splice variants or transcript forms. According to transcriptome analysis, probiotic *Bifidobacterium longum* ssp. *infantis* ATCC 15697 strain with milk fat generated a multifaceted stress response by increasing the expression of raffinose synthesis, branched-chain amino acid metabolism, and two bile excretion transporter genes under bile stress [90]. To evaluate the genetic responses of probiotic *Limosilactobacillus reuteri* strain to environmental factors and physiological conditions, independent component analysis (ICA) was applied to the dataset in ML algorithms for transcriptional data, and upregulation of arginine metabolism, riboflavin, and fatty acid synthesis genes was determined [9]. Thus, it appears to have the potential to predict phenotypic outcomes such as environmental tolerance or host interaction potential from transcriptomic patterns by AI.

Following transcriptomics, metatranscriptomics has recently attracted interest as a new omics approach that enables comprehensive analysis of active gene expression profiles within complex microbial communities such as the gut microbiota without the need to culture individual species [91]. Unlike traditional transcriptomics, which focuses on a single species of organism, metatranscriptomics examines the real-time functional activities of all microorganisms in an environment by sequencing total RNA. This approach provides important information about metabolic activities, stress responses, and interspecies interactions occurring in probiotic applications [92,93]. Through this analysis, gene expression

intensity and differential expression levels can be determined, enabling the identification of interactions and dynamic changes between microorganisms and the host. In the case of probiotics, metatranscriptomic analyses have also been utilized to examine how strains modulate gene expression patterns in native microbiota and transcriptionally adapt to intestinal environmental stress. Peng et al. [91] evaluated the active gene expressions of probiotic strains in the host with metatranscriptomic data obtained from human and animal intestinal microbiota and revealed that some probiotics showed a significant negative relationship with pathogens such as *Salmonella* spp., *Mycoplasma* spp., and *Escherichia coli*. Wang et al. [92] used metatranscriptomic analysis to examine the survival, growth, and gene expression dynamics of the orally administered probiotic *L. casei* Zhang in the human intestinal environment. The findings determined that the expression of sRNA responsible for growth and lactic acid production decreased after the probiotic entered the human GIT. Integration of metatranscriptomic data with AI-based models appears to be an important strategy to predict dynamic changes in microbial communities and probiotic viability. However, despite this potential, no studies have been found in the literature on the use of metatranscriptomics in AI-based probiotic research.

Proteomics deals with the analysis of protein properties and structures. The data obtained in this field are usually obtained by mass spectrometry (MS) techniques (LC-MS/MS, LC-QTOF/MS, MALDI-TOF, etc.), and the mass/charge ratio, peptide fragments, and amino acid sequences of proteins are determined. Proteomic approaches are effectively used to identify proteins involved in the response of probiotics to environmental conditions to understand their behavior in the food matrix and their survival capacity in the host intestine, and to detect protein-level modifications that may affect probiotic functionality [94]. The proteosurfaceome, which consists of proteins on the bacterial surface, plays a key role in the communication of probiotics with the host and has therefore been extensively studied with proteomic approaches to elucidate the functions of these proteins in gastrointestinal adaptation and probiotic effects [95]. These proteins are critical for adhesion to intestinal epithelial cells. Specific peptide profiles were obtained as a result of MS analysis of adhesin-like proteins found on the surface of *Lactobacillus plantarum* strains [96]. It has also been determined that the developmental phases of probiotics can be determined through proteomic analysis, and this is directly related to probiotic functions [97]. AI algorithms can identify functional patterns from the amino acid sequences of these proteins or predict unknown protein structures. In particular, convolutional neural networks (CNNs) have been reported to offer promising results in terms of protein structure-function prediction [98]. Moreover, by comparing proteomic profiles emerging under different conditions, adaptation mechanisms of strains to environmental stresses can also be deciphered.

Besides proteomics, metaproteomics analyzes the collective proteome of multiple organisms within an environment, such as the gut microbiota, providing a comprehensive understanding of microbial activities [99]. Metaproteomics is thus a robust tool that can facilitate the large-scale identification and quantification of proteins in complex microbial communities, offering insights into their functional states. In a typical metaproteomic workflow, proteins are extracted from the sample, enzymatic digestion (optional), separated by high-resolution LC, and subsequently analyzed using MS systems [100]. The resulting complex peptide spectra are then matched to extensive databases to identify proteins and determine their functions. Generated data include peptide counts, protein abundances, post-translational modifications, and taxonomic affiliations. The incorporation of metaproteomic data in probiotic research offers functional profiling of microbial communities and their interaction with the host [101]. Metaproteomics helps to understand how probiotics are able to survive under gut conditions, compete with pathogens, and alter the immune response of the host. Metaproteomics, hence, bridges genomic capacity and

actual phenotypic output. In addition, metaproteomic analyses can evaluate the effects of probiotics on health based on markers of functional properties. Rueangsri et al. [102] identified proteins that could be antioxidant markers in LAB-fermented vegetables by metaproteomic analyses. It has been reported that earlier diagnoses can be made about human health by determining markers as a result of fecal metaproteomic analysis with various ML models [103]. Therefore, it would be promising to combine AI techniques with metaproteomic analysis of probiotics to obtain faster information about a specific health relationship or metabolic activity in a mixed microbiome environment. Despite this potential, the applications of AI to metaproteomic datasets in the context of probiotics have not yet been illuminated by research studies in the literature. Among meta-omics technologies, most AI-supported microbiome studies still focus on metagenomic [104] or metabolomic [105] data rather than functional proteomic markers.

Lipidomics is one of the omics fields that covers the qualitative and quantitative analysis of all lipid compounds in cells. The membrane lipid composition of probiotic microorganisms plays a critical role in their adaptation to environmental stress factors and their interactions with the host organism [106]. In lipidomic analyses, high-resolution chromatographic techniques such as shotgun MS, GC-MS, and LC-MS are generally used [107] to identify different lipid classes such as phospholipids, glycolipids, and sterol derivatives. Additionally, lipidomic analyses provide comprehensive information on lipid classes, molecular shapes, chain lengths, degrees of unsaturation, and the overall fatty acid composition [108]. Lipid profiles of the cell membrane of *Lactobacillus coryniformis* Si3 showed alteration depending on pH and temperature stress, decreasing lipid saturation, and these changes have been attributed to cell survival [109]. The resulting lipidomic data are usually in the form of dense numerical data (e.g., concentration per lipid species in μg/mg cells), therefore multivariate data analysis is inevitable. Schifano et al. [110] compared the phospholipid profiles of the same probiotic product supplied by two manufacturers by lipidomic data analysis and demonstrated significant differences in the phosphatidylcholine and phosphatidylglycerol lipid classes, indicating that the probiotic properties can be influenced by different manufacturing conditions. Additionally, the authors revealed that such variation can also alter the probiotic effect on the host organism. Therefore, by classifying lipid profiles, AI can provide information about environmental relationships and probiotic properties. However, the integration of lipidomic analyses with AI in the evaluation of probiotics remains quite limited in the literature, indicating the need for further research in this area.

Metabolomics is a field that studies the molecules synthesized or used in the environment by probiotics. These data are direct biochemical results of phenotypic traits. Metabolite profiles, for example, SCFAs (acetate, propionate, butyrate), lactate, vitamins, or amino acid derivatives are determined by nuclear magnetic resonance (NMR) and GC-MS/LC-MS techniques [111]. *Bifidobacterium adolescentis* has been reported to show differences in SCFA production profiles according to the carbon source, and these differences are revealed by metabolomics data [112]. Metabolite formation and differences in their abundance in various probiotic lactic acid bacteria and bifidobacteria strains have been determined and associated with their different metabolic pathways [94]. With these analyses, metabolite data are obtained numerically, and AI algorithms can learn patterns associated with health effects from these numerical data. In this regard, a correlation can be established between a specific SCFA profile and anti-inflammatory effect, and strain selection can be made accordingly. In a study examining individuals supplemented with *Bifidobacterium longum* BB536, metabolomic data from fecal samples were integrated with AI to predict individual responses, and it was shown that levels of SCFAs, especially propionate and butyrate, increased significantly in individuals [105]. However, among

omics technologies, there are limited studies in the literature in which metabolomics data of probiotics are coordinated with AI. It is anticipated that further developments will occur over time by closing the gap in this area. Thus, the role of probiotics in diseases can be revealed more clearly with AI technologies.

The metagenomic approach examines microbial relative abundance by sequencing the 16S rRNA sequence using next-generation sequencing [90]. Metagenomic analyses can be performed specifically to assess bacterial abundance or presence in probiotic products [113]. Besides, these analyses can examine how probiotics behave in the natural microbiota (in foods or in the intestines), their interactions with other microorganisms, and their functional contributions at the community level. Studies using 16S rRNA sequencing and shotgun metagenomics techniques can show which bacterial groups are increased or decreased in the gut microbiota by certain probiotic interventions [114]. It has been reported that probiotic *Enterococcus durans* EP1 interventions cause an increase in the abundance of *Faecalibacterium prausnitzii*, which is among the new-generation probiotics and has anti-inflammatory properties [115]. Probiotic supplementation is evaluated as clinically positive by increasing the number of functional bacteria in the intestinal microbiota. Metagenomic data are obtained in the form of OTU (operational taxonomic unit) tables and abundance profiles. AI systems may be a potential tool, especially for developing microbiota-based personalized probiotic recommendations. AI models processed with metagenomic data can be used to select probiotic strains appropriate to the individual microbiota [86].

Overall, this section highlights the significant contribution of omics technologies in providing a holistic overview of probiotic characteristics, environmental adaptations, and functional abilities. Moreover, the integration of these complex datasets with AI technologies is also pointed out as a potential strategy for probiotic studies. Table 2 presents a systematic overview of the interfacing between various omics technologies and AI tools in probiotic science.

Table 2. Integration of omics technologies and AI applications for probiotic science.

Omics Approach	Data	Evaluation Output	Application Area	AI Tools	Reference
Genomics	Whole sequence, DNA sequences, annotated genes	Identification of functional genes, prediction of probiotic traits	Strain selection, probiotic characterization	Machine learning, deep learning, natural language processing	[13,116]
Transcriptomics	Raw RNA reads, single-cell RNA-seq data, mRNA expression levels, etc.	Gene expression profiling under stress, prediction of stress responses	Strain selection, probiotic characterization	Machine learning and clustering algorithms (such as hierarchical clustering or K-means)	[9]
Metatranscriptomics	Total RNA expression profiles in microbiota, RNA-seq data	Functional activities of probiotics within microbiota, host interaction	Strain selection, probiotic characterization, personalized diet, formulation	Machine learning, deep learning	[116]
Proteomics	Proteins, peptide sequences	Protein structure-function prediction, adaptation analysis	Viability, strain selection, functional food design, disease diagnostics, drug development	Machine learning, deep learning, natural language processing	[13,116]
Metaproteomics	Collective protein profiles, functional markers	Functional protein markers linked to probiotic activity	Health biomarker identification, survival	Machine learning, deep learning	[117]

Table 2. *Cont.*

Omics Approach	Data	Evaluation Output	Application Area	AI Tools	Reference
Lipidomics	Lipid composition, lipid profiles	Membrane lipid profiling, adaptation indicators, host interaction	Strain selection, probiotic characterization	Machine learning, deep learning, natural language processing	[13,116]
Metabolomics	Metabolite concentrations, metabolic fingerprints	Metabolite profiles (e.g., SCFAs, vitamins), prediction of health effects	Strain selection, functional food design, personalized diet	Machine learning, deep learning	[13,118]
Metagenomics	Microbial abundance tables (OTUs, taxa profiles)	Microbial abundance shifts	Strain selection, microbiota composition optimization, functional food design, functional food design	Machine learning, deep learning (such as Meta-Signer, DeepMicro, mAML, PaPrBaG, MicrobiomeAnalystR, mothur, QIIME, BiomMiner, Scikit-learn, and MIPMLP)	[119]

3.2. Mechanism of Action

To understand whether a microorganism is probiotic, in vitro and in vivo tests must be performed. DNA/RNA-based genotypic diagnostic methods, which are faster and more reliable, are used. PCR-based methods such as PCR, 16S rRNA sequence analysis, and real-time PCR [120]. Figure 1 shows a diagram of the AI-driven workflow for microbial genomic analysis.

3.2.1. Role of AI on Microbiota Modulation

The human gut microbiome has been called the second genome [121]. In recent years, the combination of microbiome science and AI technologies has ushered in unprecedented growth in health and biotechnology. Relative to sluggish and non-interpretive traditional microbiome analysis methods, AI-based methods are immensely useful for dealing with large sets of data, disease forecasting, and designing bespoke health interventions [122]. AI technologies including ML [12], DL, natural language processing (NLP) [123,124], computer vision (CV) [124], reinforcement learning (RL) [125], robotic process automation (RPA) [124], generative AI (GenAI) [126], Edge AI [127], and quantum AI (QAI) [128], as well as their applications in healthcare [124], autonomous systems, and AI ethics, are increasingly being used to interrogate complex microbiome data and accelerate our understanding about microbial relationships and their translation for human disease [122,129]. All these AI technologies and their interactions are illustrated by bibliometric analysis as in Figure 2. AI-driven methods accelerate the discovery, formulation, development, and optimization of probiotics for target microbiomes, enabling more efficient and personalized interventions. The convergence of AI and microbiome science holds potential for the treatment of gut health and precision medicine interventions [125].

Probiotics must survive under harsh conditions, such as bile salts and stomach acid. The goal is to preserve gut microbiota and attain maximum probiotic effectiveness. Active ML enables effective microbiome analysis via the choice of relevant features, identification of biomarkers, disease prediction, and treatment suggestion, and enhances precision with scarce data [14,86]. ML is divided into supervised learning and unsupervised learning. Supervised learning involves the prediction of continuous variables and the classification

of data into categories. Unsupervised learning is employed to group samples into clusters based on similarity. Data preparation and collection, ML model training, and model performance are all involved in model development. Performance of the model is measured based on accuracy, precision, recall, and the ROC curve [122]. ML expedites microbiome research with drug interaction prediction, microbiome therapy design, and optimization of personalized medicine. Advances in AI also reveal gut microbiota, allowing for more efficient and targeted treatment [130,131]. It is necessary to combine AI and microbiome science to develop personalized probiotic therapy. AI processes large NGS datasets efficiently, revealing critical information about gut microbiome function and structure, which helps in the choice of active probiotic strains [117]. In ML, one must strike a balance between underfitting and overfitting, and models must be optimized to deliver maximum performance. Unoptimized algorithms will overfit the training set and not generalize and will miss out on detecting patterns in data [131]. To prevent overfitting and underfitting, techniques such as data augmentation, controlling model complexity, cross-validation, early stopping, regularization, and opting for simpler models can be employed.

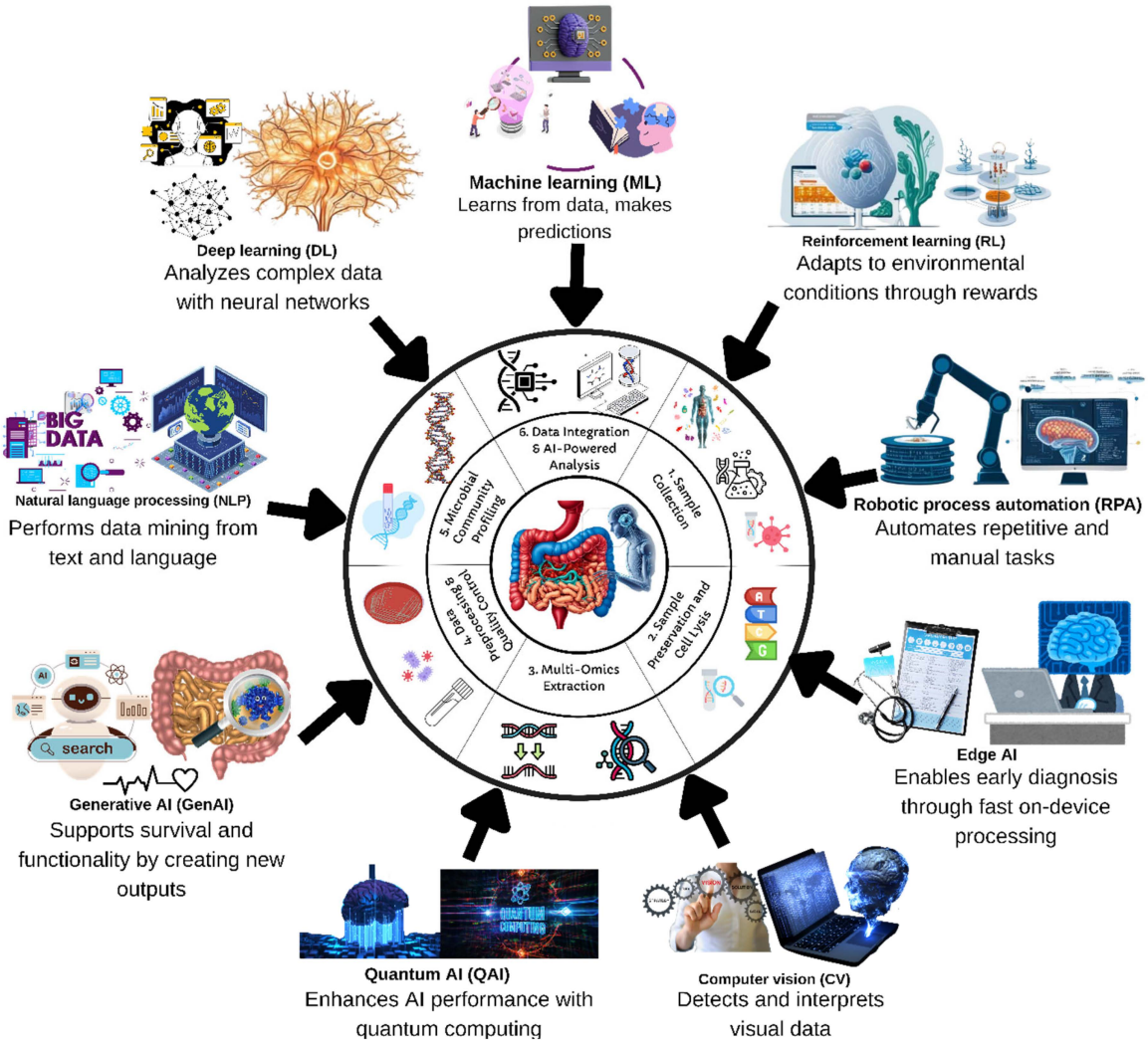


Figure 1. The integration of advanced AI technologies, including ML, DL, NLP, CV, RL, RPA, GenAI, Edge AI, and QAI, accelerates probiotic research. These approaches facilitate complex data analysis, environmental adaptation, early diagnosis, automation of manual tasks, data mining, multi-omics integration, and the generation of novel functional insights to enhance the understanding and application of probiotics.

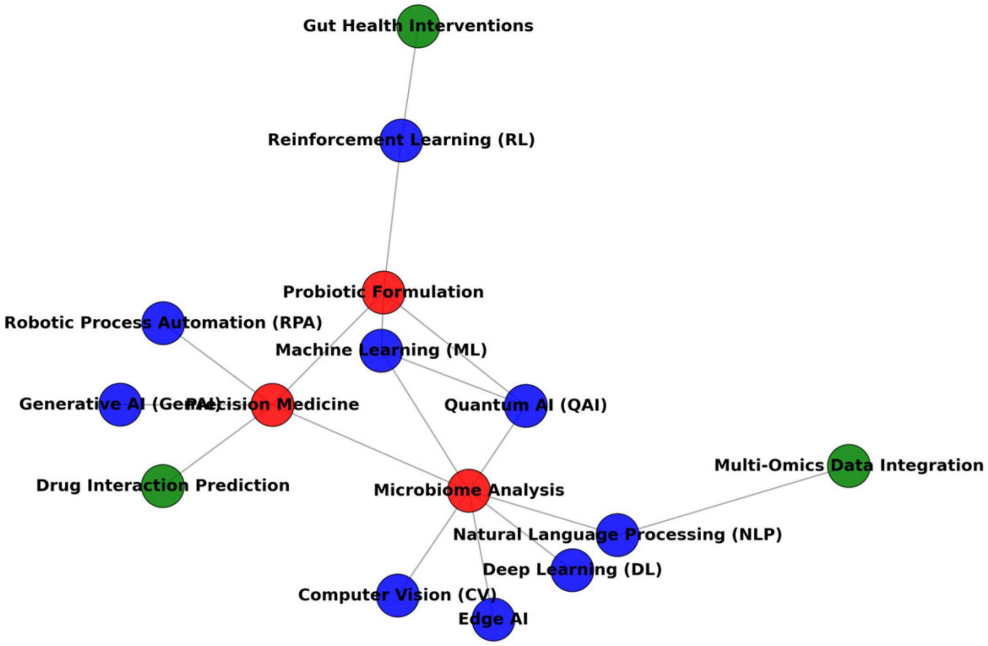


Figure 2. Bibliometric analysis between AI technologies and human gut microbiota. Red dots represent microbiome formation (e.g., microbiome analysis, probiotic formulation, precision medicine); blue dots represent the AI and data processing technologies used (e.g., computer vision, natural language processing, deep learning, machine learning, etc.); and green dots represent targeted applications and outcomes aimed (e.g., gut health interventions, drug interaction prediction, multi-omics data integration). The network architecture highlights cross-domain interactions and multidisciplinary integration.

DL methods, including CNN and recurrent neural networks (RNNs), are of special significance in time-series microbiome data analysis, microbial interaction prediction, and identification of major differences between healthy and diseased patients. By applying ML models to high-throughput sequencing data [12], such as whole-genome sequencing and 16S rRNA sequencing, researchers can uncover patterns of gut microbiota composition and probiotic efficacy association [86,132]. DL models are also used for the detection of intestinal parasites from fecal samples and bacterial colony morphology analysis using CNN. Additionally, CNN models applied on mobile devices also enhance efficiency in the detection of pathogens and rapid diagnosis. Prediction of antibiotic resistance genes has also been performed using deep learning-based genome analysis [122].

NLP is a technology that processes text to extract information from large datasets and is a powerful tool for analyzing functional food research [133]. NLP, data mining, and identification of relevant biosynthetic gene clusters can be carried out by focusing on higher-order information and gene vicinity. Mapping NLP technologies onto a biological model is an efficient way of "standardizing" the intuitive extraction process [134]. Thus, text-mining algorithms can provide researchers with comprehensive information on microbiota modulation by helping to identify microbial interactions, functional pathways, and trends in probiotic applications.

CV techniques are widely used in microscopy and imaging to analyze bacterial morphology. It identifies and analyzes bacteria using computer algorithms. CV has the advantage of being automatic, objective, and fully reproducible. It observes data that cannot be detected by human experience, allowing us to get precise results [135]. In a study, cheese images obtained with an ordinary camera are processed with CV techniques and then classified using relevant ML algorithms [136]. AI-powered imaging tools can help visualize gut microbial changes, allowing us to drill down and evaluate the effects of probiotics.

RL is the process of observing environmental conditions and choosing from a structure that changes the state of the environment, and detecting the behavior of the environment [137]. RL focuses on decision making by rewarding desired outcomes for the microbiota [125]. RL can deliver personalized probiotic treatments based on real-time microbiome data through AI-driven feedback loops and rewards.

RPA is a piece of software that mechanizes most of the routine work and tasks that require human involvement to automate business processes. RPA is a distinct type of technology from robotics [138]. RPA can accelerate data analysis, production processes (such as strain detection), quality control, and optimization of probiotics.

GenAI is a technology used to analyze large text datasets, identify patterns, and generate content. AI can also create virtual instructors that offer customized instruction and feedback by mimicking one-on-one interactions with human instructors [139]. GenAI can study probiotics with improved survival and functionality in the gut environment and provide insight into their effects.

Edge AI facilitates real-time decision making for applications ranging from earliest disease detection in healthcare to infectious disease spread tracking and wearable device-based patient monitoring. It refers to an AI technology that performs data processing at the edge instead of a cloud-based centralized infrastructure [127].

Advanced imaging techniques supported by quantum sensors and AI analysis can diagnose diseases at earlier stages, provide timely interventions, and deliver faster results. It can analyze a person's specific genetic makeup and disease profile and adjust personalized treatment plans with fewer side effects. The synergy between AI and quantum computing holds great promise for drug discovery. In the healthcare sector, biosignals play a major role in diagnosing certain disorders [128]. Quantum computing could eventually increase the speed and accuracy of AI processing, enabling microbiome analysis and deep probiotic-microbiota interactions, and optimizing gut health strategies.

ML clinical trials can present some challenges. Some important factors to consider include ensuring data security and confidentiality, and handling incomplete or missing data. Genomic, transcriptomic, proteomic, lipidomic, metabolomic, and metagenomic information provide genetic, amino acid, fatty acid, metabolite, and microbial profiles, respectively. These are integrated with AI and network analysis software in Figure 3 and are used for disease diagnosis, targeted probiotic design, functional probiotic screening (e.g., antimicrobial peptide production, bile tolerance, mucosal adhesion), personalized dietary recommendations, drug-probiotic interaction modeling, and analysis of effects on global microbiome diversity. AI-assisted optimization enables next-generation probiotic development strategies.

Active ML predicted the effect of pharma excipients on *Lacticaseibacillus paracasei*. Despite the small dataset (accuracy rate 67.7%), it was shown that ML can be effective for probiotic formulation design [14]. Optimal candidates were determined after screening the probiotic and antimicrobial activities of 144 LAB strains using ML algorithms. Implications of drug excipients on *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, and *Lacticaseibacillus casei* were explored under active learning, and ML was found to be effective even with small datasets [43]. AI models have been applied to develop tailor-made probiotic formulations by translating big data in human microbiome studies [140]. It was confirmed that *Limosilactobacillus reuteri* strains improve intestinal health in calves and the relationship between intestinal microbiota and health condition with ML-supported models [141]. Table 3 shows key studies using various AI techniques, highlighting their findings and implications for probiotic research.

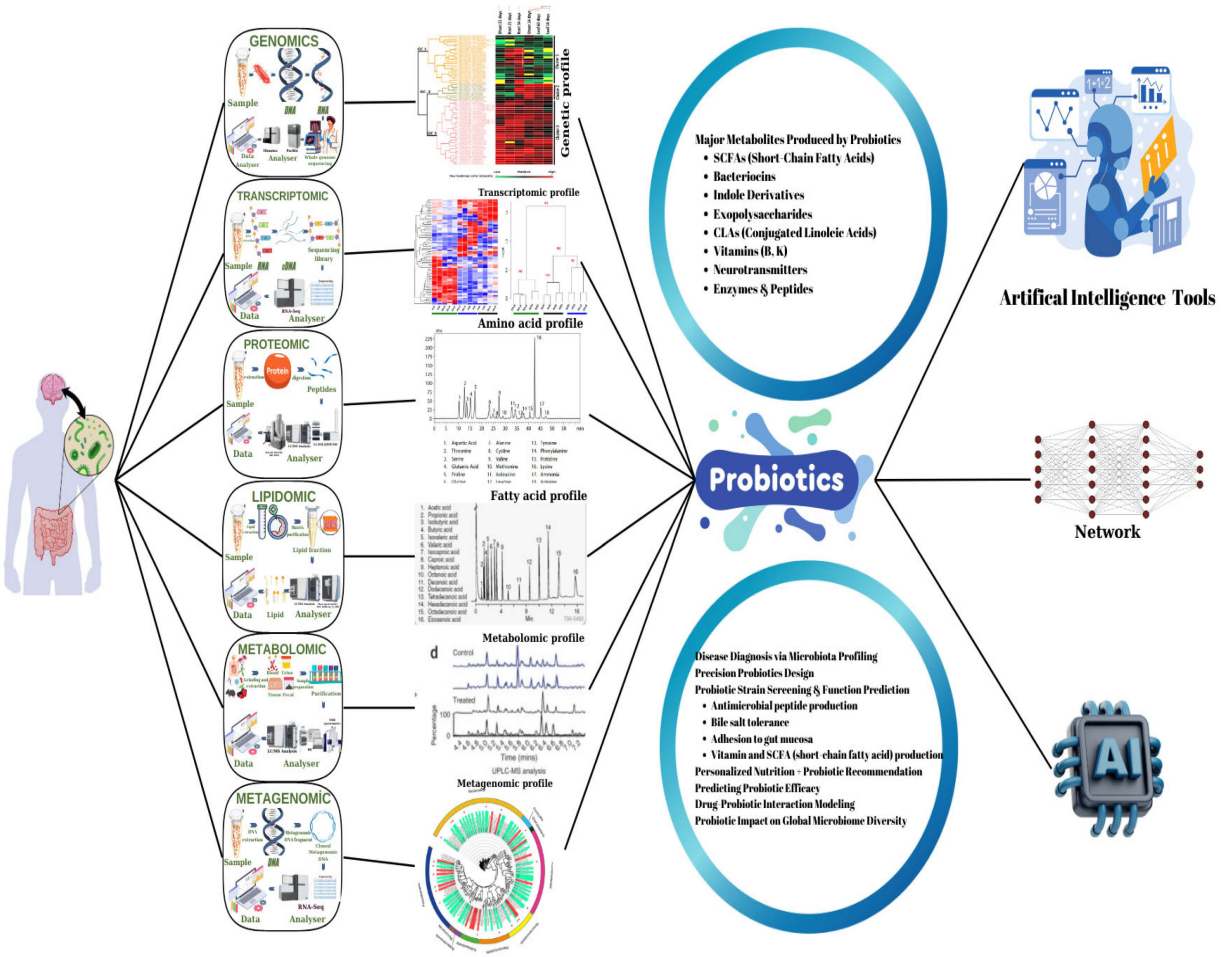


Figure 3. Integration of multi-omics data with AI tools in probiotic research and its application areas. On the left side, omics data sources (genomics, transcriptomics, proteomics, lipidomics, metabolomics, and metagenomics) from which biological information about probiotics is obtained are located. Each omics layer provides a detailed profile of the metabolites produced by probiotics and their functions. The middle section shows the molecular-level outputs obtained from these layers. On the right side, the analysis of these multi-dimensional data using AI and machine learning methods and their transformation into disease prediction, personalized probiotic design, and microbiota-based applications are visualized.

Table 3. Use of AI in probiotic research and results.

Key Findings	AI Technology	Significance	Reference
PCA used to visualize excipients' chemical structure impact. The model started with 6 excipients, predicted effects on 111 ones. After 3 rounds of AML, achieved 67.7% accuracy, identifying 3/4 tested excipients.	Active ML to predict the effects of pharmaceutical excipients on *Lacticaseibacillus paracasei*.	Demonstrates ML's power in pharmaceutical formulation even with small datasets.	[14]
144 LAB strains were evaluated for low pH, bile salt resistance, and antimicrobial activity. Best strains identified: *Lacticaseibacillus paracasei* S23, *Lactiplantibacillus plantarum* S57 & S70, *Lacticaseibacillus casei* S81. Decision Tree algorithm validated biofilm production's role in gut colonization.	Applied ML algorithms: Information Gain Ratio, Information Gain, PCA, Gini Index, Chi-square, Deviance, Rule-Based Learning, Uncertainty, Correlation, and Relaxation.	ML methods successfully identified top LAB strains with >99% accuracy.	[43]

Table 3. *Cont.*

Key Findings	AI Technology	Significance	Reference
AI used to identify beneficial microbial species, link them to diseases, and develop personalized probiotic formulations. NGPs could be key to personalized medicine.	AI-driven data analytics for large-scale gut microbiota studies across geographic regions.	AI-microbiome integration could revolutionize clinical applications and personalized probiotics.	[140]
91 calf microbiome samples (74 healthy, 17 diarrheic used to train random forest model. *Limosilactobacillus reuteri* administration restored gut microbiota in diarrheic calves. Model identified health-associated bacteria.	Whole-genome sequencing of 22 *Limosilactobacillus reuteri* strains; random forest model to analyze gut microbiome profiles.	Probiotic treatment confirmed effective in restoring gut health; ML model validated findings.	[141]

In the future, microbiota can be considered as a system that can change its chemical structure according to factors such as body temperature, stress level, and disease status, and communicate with the mind through bacteria equipped with a neural network [142]. Intestinal microbiota can be monitored instantly with rapid tests that can be performed in the home environment [143]; thus, it will be easier to follow diseases such as IBS, Crohn's disease, and ulcerative colitis [144]. It will be possible to develop NGP drugs through AI-supported production systems [145]. Treatment effectiveness can be increased with probiotics, with increased adhesion using genetic engineering techniques, and nanorobots that perform targeted release [146]. Individual-specific microbiota tracking can be made possible thanks to wearable biosensors, smart capsules, and sensor food packaging [147]. Using metagenomic analyses and ML, personalized probiotic matches can be made, the effects of the gut–brain axis can be better understood [148], and drug interactions can be predicted [119]. With all these developments, microbiota can become a system that predicts diseases in the body, processes biological information, and functions as a health assistant.

3.2.2. Role of AI on Metabolite Production

The gut microbiota is a complex ecosystem associated with human health and disease. AI and ML methods have been increasingly used to explore the composition and function of the gut microbiota in recent years. Predicting the effect of probiotics on the gut ecosystem is among the biggest microbiome research tasks. AI-assisted data analysis allows for the identification of important microbe species, functional genes, and metabolites associated with some health conditions [104]. Metabolites are intermediate or end products of interactions between microbes and host cells [149]. ML models predict the metabolic activity of microbial communities to determine which microorganisms produce individual metabolites and how these could affect host health. Gut microbiota plays a key role in the metabolism of nutrients, nutraceuticals, vitamins, minerals, and dietary polyphenols, and is limited primarily by their size and complexity. AI has probed the complexity of the human gut microbiota, and together with an ML algorithm for building optimized probiotic formulations, studies have demonstrated that the environment of the gut microbiota heavily impacts the growth and metabolite activity of bacterial communities [130]. AI has improved the ability to predict the production of different microbial secondary metabolites of pharmaceutical and industrial significance by different probiotic types. Metabolite analysis assisted by AI enhances fermentation technology and probiotic function by identifying notable probiotic metabolites such as antimicrobial peptides (AMPs), SCFAs, EPS, and phenolic compounds (PCs). Case studies also demonstrate that AI models achieve over 97% accuracy in the recognition of bacteria and accelerated metabolite discovery. It cures

the side effects of antibiotics and strengthens the host's health by producing antimicrobial metabolites. AI manages the probiotics' EPS production and optimizes the amounts of PCs, and accordingly increases efficiency while reducing resource expenditure [21].

In the study, the interaction of probiotics and intestinal microbiota and their synergistic effects on metabolite production were evaluated using the ABIOME AI model, and the most severe interaction was determined in the *Bifidobacterium longum* and *Ligilactobacillus salivarius* pair [104]. By comparing the genomic information of 239 probiotic and 412 non-probiotic bacteria, a 97.77% accuracy rate was obtained using the support vector machine (SVM) algorithm (ML algorithm), and it was shown that probiotic characteristics can be predicted [150]. By classifying 89 bacteria using various ML models, it was observed that the best accuracy was 95.1% in ANN [151]. Through transcriptional regulatory network analysis, significant gene regulatory mechanisms were found to be optimized for probiotic traits. All such studies are elaborated in Table 4.

Table 4. Use of AI in probiotic for metabolite production.

Key Findings	AI Technology	Database Used	Applications	Reference
Applied ABIOME model to the optimization of probiotic formulation; used MARS algorithm; reported synergistic interactions in metabolite production.	ML algorithm for probiotic synergism	ABIOME bioreactor, MARS algorithm, probiotic-metabolite interactions	Development of next-generation probiotics, personalized formulations	[104]
Developed machine learning model (SVM) to predict probiotic potential based on genomic k-mer analysis; achieved 97.77% accuracy.	Support vector machine (SVM) algorithm model	Genomic k-mer analysis, SVM model, incremental feature selection	Probiotic genome classification, food and supplement industry applications	[150]
Analyzed 89 bacterial genomes; applied multiple ML models; neural networks achieved 95.1% accuracy in probiotic classification.	Multiple ML models (GLM, RF, SVM, NN) are used to predict the ability to classify bacteria	NCBI GenBank data	Probiotic identification for food and health industries	[151]
Used ICA to analyze Limosilactobacillus reuteri transcriptional regulation; identified 35 iModulons; discovered bistable regulatory mechanisms.	ML model	RNA-seq datasets, independent component analysis (ICA)	Optimization of probiotic properties, microbial food production strategies	[9]

3.2.3. Role of AI to Understand Immune Modulation

Probiotics are defined as bacterial molecular effectors that affect immunomodulatory mechanisms together with other non-protein molecules such as bacterial proteins, lipoteichoic acids [152], teichoic acids (TAs), EPS, and peptidoglycan (PG) [153]. Probiotics can interact directly with immune cells through their surface molecules, secrete anti-inflammatory cytokines, and promote the differentiation of Treg cells [154]. Current AI advances open possibilities for immunology to gain a deeper understanding of the human immune system. AI systems leverage their ability to integrate existing knowledge from a variety of sources and data formats, including biological, clinical (e.g., electronic health records), and wearable device information at the molecular to patient level. AI algorithms will enhance the timely detection of patient reactions to immunotherapies and reveal biomarkers affecting immunotherapy resistance. But issues like data quality, explainability of models, bias, validation challenges, and expenses need ethical and sustainable use [154].

3.2.4. Models and Algorithms in AI-Assisted Probiotic Research

Understanding the nature of the gut microbiome is important for disease prediction. Genomic data allow monitoring of microbial community changes in the gut flora. However, the analysis of these data is complex and new techniques such as AI/ML can help process large datasets quickly and efficiently [155]. For AI-supported probiotic research, various types of input data are primarily required, including larger sets of omics (multi-omics) data [156], large-scale probiotic data collection, and mining using DNA data [157]. Probiotic research employs both structured (e.g., numerical clinical datasets) [158] and unstructured data types (such as images, text, or in this case, microbiome sequencing data) [86]. In the data preprocessing phase, activities such as cleaning missing values, normalization, feature selection, dimensionality reduction, and data augmentation are performed. The computational cost varies depending on the complexity of the model used and the performance of the model is evaluated using cross-validation or holdout datasets in terms of accuracy, precision, and sensitivity [159,160]. Decreasing production costs of omics data are supporting the development of machine learning models; however, the integration of multi-omics data and elucidation of biological interactions still pose significant challenges [161].

In order to accelerate the time-consuming and costly processes required to investigate the relationship between probiotic strains and diseases, studies are being carried out with high accuracy and efficiency in areas such as strain selection, functional effect prediction, biomarker discovery, and disease risk analysis using AI-based methods [162]. The increasing use of artificial intelligence and machine learning models carries the risk of healthcare providers facing the burden of collecting large amounts of data. Therefore, it is important to determine them as cost-effective and informative. CoAI is an ML model that allows healthcare providers to make more accurate predictions with less data. CoAI enables more accurate clinical risk predictions by reducing data collection costs in healthcare. The model selects low-cost, high-information features to provide more effective predictions than existing clinical scores [163]. Also, model-based strategies play an important role in probiotic strain design. These strategies provide organized information on biochemical reactions and metabolites using genome-scale metabolic models (GEMs). Integration of omic data can make the design of probiotic strains more accurate, because engineering decisions can be made more soundly with information at the cellular level. Furthermore, the long-term stability and therapeutic efficacy of smart probiotics can be increased by considering the resistance of probiotics to environmental factors and their metabolic load. These approaches aim to improve the safety and production capacity of probiotics [164].

4. Precision of Health Effects

AI will find a place in many areas such as disease diagnosis, probiotic optimization, and personalized medicine. AI systems will be able to analyze large-scale microbiome data to identify microbial biomarkers associated with diseases such as cancer, autoimmune diseases, metabolic syndrome, and GI diseases. This will boost nonsurgical diagnostic approaches and improve the efficiency of disease management. Microbiome analysis and AI will gain prominence in the coming years [122].

4.1. Intestinal Health

Certain probiotics have shown benefits in certain types of GI diseases, and interact with the GI ecosystem by accelerating GI transit and reducing the ability of disease-causing bacteria to colonize and adhere to the GI mucosa. Among them; probiotics might have roles for IBS, *Helicobacter pylori* eradication, inflammatory bowel disease, diarrhea, GI disorders, allergic diseases (e.g., atopic dermatitis), nonalcoholic fatty liver disease, obesity, insulin

resistance syndrome, type 2 diabetes, different types of cancer and cancer-related side effects, immune health, metabolic health, dental health, and brain health [152,162].

The gut microbiome is unique and may be dynamic with disease, so it is a great tool for disease prediction and biomarker discovery without prior knowledge of ML models [149]. The incorporation of AI in gut microbiome research enhances the absorption of nutrients, immune homeostasis, and intestinal barrier function. AI probiotics strengthen the intestinal mucosa, restore microbiota, and reduce inflammation. ML gut-on-a-chip models efficiently screen the probiotic–gut cell interaction. AI platforms also incorporate microbiome data with medical records and biosensors to enable real-time management of health and prevention of disease [21]. AI-powered algorithms can be applied to enhance probiotic treatments via the combination of clinical data and microbiome profiles. ML algorithms can foresee personalized probiotic treatments based on the microbiome composition and clinical characteristics of an individual. However, the application of AI in medicine must be carried out safely, equitably, and transparently, with consideration of ethical and regulatory factors [86].

In studies on the microbiome, high accuracy rates have been achieved in areas such as disease classification and biomarker discovery using machine learning algorithms such as SVMs (support vector machines), RFs (random forests), Lasso, and ENet. In addition, deep learning methods allow more information to be extracted from microbiome data by analyzing more complex datasets. The application of these artificial intelligence techniques has yielded very successful results, especially in health status monitoring and early disease prediction of microbiome data [165].

Another study is to develop a system to evaluate the effects of probiotics on irritable bowel disease (IBD) using intelligent intestine-on-a-chip technology. This system includes a scalable intestinal microchip that enables high-throughput co-culture of intestinal cells and microbes, and an ML-based analyzer to measure therapeutic differences between probiotic strains. This approach promises to offer a new platform for the identification of probiotics or synbiotics using microfluidic chips and ML [166].

In another study, ML techniques were used to diagnose intestinal inflammatory diseases (IBDs), especially to distinguish between Crohn's disease (CD) and ulcerative colitis (UC). The sPLS-DA (sparse Partial Least Squares Discriminant Analysis) model was applied in the analysis of the data. This model can effectively manage redundant or repetitive variables in high-dimensional data and reveal the importance of certain microbial species in disease status prediction. The findings of the study show the potential of microbial biomarkers that can distinguish IBD and healthy control groups with high accuracy rates. In addition, distinctive microbiome profiles were determined between CD and UC, and it was concluded that machine learning models offer a promising approach for the diagnosis and differentiation of IBD [167].

4.2. Anticancerogenic Activities

AI and deep learning algorithms are used in image analysis (scans such as MRI, CT, PET) and biopsy samples for early cancer diagnosis, and can detect tumors much earlier and more precisely than the human eye. In addition, genomic data are analyzed to create individual tumor profiles and develop personalized treatment (precision oncology) approaches. Artificial intelligence is also effectively used in clinical decision support systems to predict the stage of cancer and the course of the disease [168,169]. In studies, the analysis of the effectiveness of microbial information to discriminate between different cancer types using ML models, and RF classifiers yielded extremely high balanced accuracy rates (87% and 96%) for HNSC, STAD, and COAD cancers, while the rate fell below 80% for ESCA and READ. In subsequent research, classification accuracy was determined by grouping cancer types into broader categories, and dimensionality reduction and over-

sampling techniques enhanced accuracy, especially for HNSC, but performed less well on STAD/ESCA classification. In other experiments with a different ML model, SHAP analysis, it was found that bacteria such as Granulicatella and Porphyromonas were important in distinguishing CRC cancers, while species such as Fusobacterium, Helicobacter, and Lactobacillus were crucial for HNSC, STAD, and ESCA. The model was, however, not able to distinguish READ from COAD and ESCA from STAD and HNSC. Although the model suggested high accuracy in cancer-type classification based on microbial data, some of the types, especially READ and ESCA, could not be classified appropriately. This was due to limitations of the richness of the data, efficacy of the methods, and learning capabilities of the model [170].

4.3. Antiaging Roles

AI techniques, such as deep learning, GAN, and network analysis, the aging process can be reconstructed in three dimensions, and potential anti-aging drugs can be screened in a virtual environment and tested with molecular dynamics simulations, greatly accelerating the drug discovery process. The multi-layered structure of human aging has become more deeply understandable with the opportunities offered by AI, which has opened new doors in the development of anti-aging strategies [171]. The main application of AI in anti-aging research is the collection of data, especially genetic and facial-based aging data. The collection of these data plays a critical role in understanding the genetic, phenotypic, and environmental factors that affect aging. Among the genetic aging databases, resources such as GenAge, AnAge, GenDR, and LongevityMap [172] contain aging-related genes in humans and model organisms. These databases are used to evaluate the effects of anti-aging treatment processes. On the other hand, other aging databases such as AnAge, DrugAge, CellAge, and Geroprotectors play an important role in understanding the aging process and the effects of anti-aging treatments [171,172].

4.4. Cardiovascular Health

The gut microbiota influences cardiac well-being through short-chain fatty acids and bile acids, and prebiotic and probiotic intake can reduce the risk of cardiovascular disease by facilitating gut-friendly bacteria development. The optimization of the relationship will provide mechanisms for defining beneficial doses and therapeutic durations of the prevention and care of such as heart failure by personalized probiotic therapy [173]. Probiotics support gut health by lipid metabolism modulation, anti-inflammation, and cardiovascular risk factor control. *Limosilactobacillus reuteri* CCFM8631 could reduce plasma cholesterol, reducing the risk of atherosclerosis. AI supports the early detection of cardiovascular disease through ECG and biomarker tests, while AI systems analyze the influence of probiotics on an individual, allowing for customized treatment [21].

4.5. Type 2 Diabetes

Probiotic supplements regulate the gut microbiota, enhancing insulin sensitivity and reducing inflammation. ML models (SVM, XGBoost) from gut microbiome profiles correctly predict T2DM risk at 70–72%. The therapeutic effectiveness of probiotic strains like *Akkermansia muciniphila* in the management of diabetes is variable based on one's gut microbiome type. AI-driven multi-omics data integration integrates gut microbiome changes end-to-end for diabetes prevention and management [21]. According to a meta-analysis by Tao et al. [174], probiotic treatment helps glycemic regulation in patients with Type 2 diabetes. In the study conducted on 902 patients, a 0.24% decrease in hemoglobin A1c (HbA1c) levels, a 0.44 mmol/L decrease in fasting blood sugar (FBS) levels, and a 1.07-point decrease in insulin resistance (HOMA-IR) were observed in the probiotic groups. These results were attributed to an improvement in glucose metabolism through modulation of

the intestinal microbiota, but a direct anti-inflammatory effect was not observed. It was emphasized that the effects of probiotics depend on the duration of application, dose, and bacterial strain, and it was concluded that probiotics may play a helpful role in the control of T2DM.

4.6. Other Roles

Probiotics may play a supportive role in weight management by modulating gut microbiota and affecting energy metabolism, appetite regulation, and fat storage. Certain strains (e.g., *Lactobacillus gasseri*, *Bifidobacterium breve*) have shown the potential to reduce body weight and visceral fat through short-chain fatty acid production and hormone regulation. However, these effects may vary significantly depending on the characteristics of the probiotic strain, the dose, and the physiological and microbial makeup of the individual [175]. Researchers can predict gut microbiome strains through the analysis of genomic structures, protein signatures, or metabolites. Such methods identify beneficial microbes and bring forth novel health solutions. AI algorithms evaluate personal information and microbial patterns, and suggest personalized treatments according to disease progression and health monitoring [117].

Studies on human epidemiology and animal models have also shown that gut microbiota may play a role in the development of autism spectrum disorders. It has been reported that probiotics can reduce neuroinflammation by balancing gut microbiota and have the potential to alleviate ASD symptoms. It analyzes brain imaging data to diagnose autism spectrum disorder (ASD) with 80% accuracy (AUC = 0.8) with AI and DL models. Additionally, ANN- and CNN-based classifiers achieved 94.5% accuracy in diagnosing ASD [21,176]. Where AI, health, and probiotics converge, a system for AI can be designed that provides personalized probiotic recommendations from a human's gut microbiome data. Such a system looks at an individual's current microbiota balance by way of metagenomic studies and ML algorithms to determine what strains of probiotics are in deficiency or in abundance and provides the best-suited blends of probiotics for specific diseases (e.g., IBS, obesity, diabetes). In this manner, both preventive health care is fostered and the effectiveness of probiotic supplements is specific to the individual.

5. Technological Perspectives with AI

The viability of probiotics is the key technological issue to ensure their functionality from the consumer's side and, most importantly, from the regulatory bodies. AI tools could be effectively used to increase the technological performance of probiotic strains mainly from a survival perspective of view (Figure 4). Probiotics must be present in sufficient numbers, alive, and functional before reaching the colon. Adequate intake of live probiotics (10^6–10^9 CFU/mL or g), also known as the minimum therapeutic level, can support human health by modifying the gut microbiota [177]. However, under various environmental stresses, the viability level of probiotics may be insufficient in the final product reaching the consumers. Environmental stress durations that probiotics are exposed to could be due to stresses during processing, storage, and digestion. The main stresses in these processes can be listed as temperature, water activity, osmotic pressure, oxidation, pH, and bile salts. In Figure 4, the key phases that determine the effectiveness of probiotic products—strain selection and interactions (Probiotics), processing conditions (Processing), storage process (Storage), and durability of digestion (Digestion)—are analyzed holistically with AI algorithms. AI integrates the learning, prediction, and optimization tasks by taking into consideration parameters such as temperature, pressure, oxidation, pH, and bile salts. This enables one to develop functional, stable, and effective probiotic products.

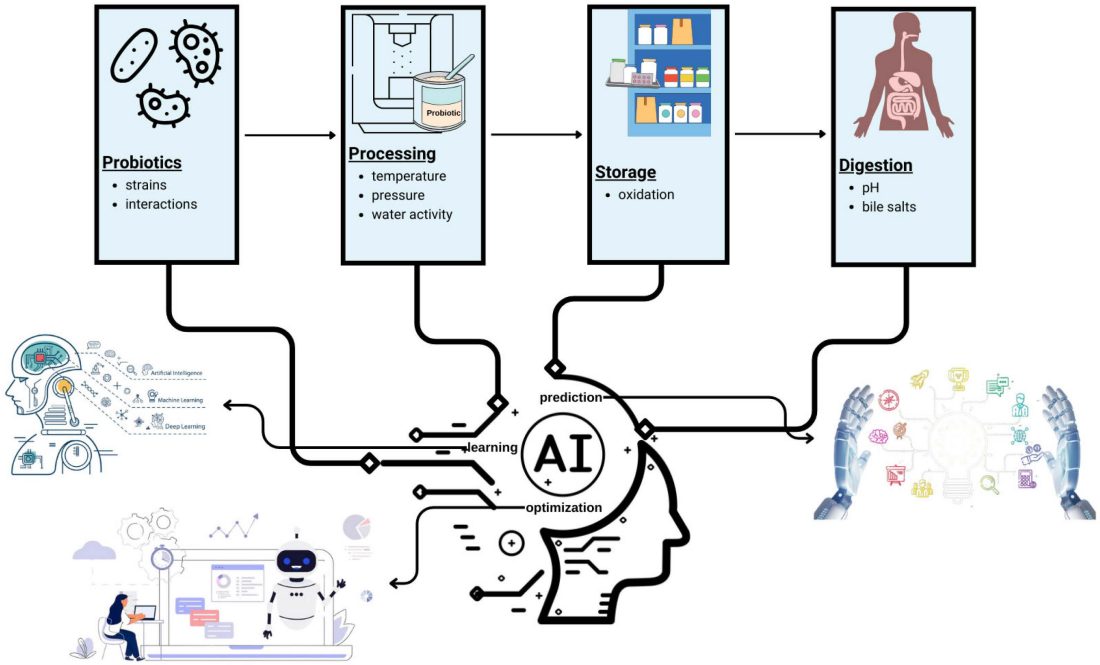


Figure 4. Integrating AI across the probiotic production chain. Using AI tools, the process conditions for the technological performances of probiotics can be predicted and optimized, and these tools could learn the final potential outcomes. The viability of probiotics, the process and storage conditions for probiotics, and finally, the digestion conditions for probiotics are targeted with AI tools to increase the techno-functional characteristics of probiotics.

Probiotic bacteria are commonly produced in dried powder form. Freeze-drying stands out as the most preferred methodology in terms of keeping probiotics viable, which are sensitive to high temperatures. However, the primary problem in preserving viability during freeze-drying is the osmotic shock that occurs due to the formation of ice crystals at very low temperatures [178]. To prevent this, the use of substances known as cryoprotectants, which prevent crystal formation by lowering the freezing point of water, is common [179]. Since a single cryoprotectant formulation is not sufficient to successfully preserve probiotics, formulations consisting of more than one component are needed, in which AI tools such as ML applications can be utilized [180]. For instance, in a research conducted by Kavak et al. [181], an experimental design was proposed for the optimization of cryoprotective media, and the optimum formulation for maximum cell survival was determined. The effects of three cryoprotectant components determined as independent variables at low and high levels were evaluated by freeze-drying measurements on three different days. Experiments were performed under 20 different conditions and random points were added to increase prediction accuracy. The most suitable levels were determined using the desirability function. Although response surface methodology [88] is traditionally accepted as a statistical optimization method, it can be considered as a part of AI-based optimization processes due to its use with AI and ML techniques in recent years. For example, Yusuff et al. [182] and Keser and Şahin [183] integrated RSM with AI techniques and used them in optimization processes. The RSM techniques used in this study can be evaluated within the scope of AI-based optimization tools. Similarly, cryoprotectant formulas used to increase the survival rate of *Levilactobacillus brevis* ED25 bacteria during the freeze-drying process were optimized using the Box–Behnken experimental design. Accordingly, the independent variables were determined as skim milk, lactose, and sucrose, and the dependent variables were determined as the survival rate after freezing and freeze-drying. At the end of the experiment, a second-order polynomial model equation ex-

plaining the relationship between the independent variables and survival rates was created, and how the cryoprotectant formulas affected the survival rate was shown [184]. Since the freeze-drying process takes place under low temperatures and pressure, it preserves vitality and provides higher quality foods in terms of nutrition and sensory. However, this process is a long-lasting and energy-consuming process, and optimizing temperature and pressure parameters is critical for maintaining energy efficiency and product quality [185]. For example, optimization problems in dried yogurt products and the best drying regime for yogurt were determined. For this purpose, the Utopia Point Optimization method was used to find the best combination of drying temperature, pressure, and time parameters. In this method, the points where each target function is best are determined and the best point found is accepted as the best solution called the utopia point. In cases where a common point cannot be found, a solution that balances all targets is determined using the S(x) mathematical method. As a result of the study, when the determined optimum freeze-drying conditions were applied to yogurt, the preservation rate of probiotics was determined as 69.291% [186]. In another study conducted by Zhu et al. [187], an ANN model called multi-task convolutional self-attention network (CSAN) was developed to determine the drying conditions that maximize the survival of probiotics. Thanks to the ability of CSAN to learn both short-term and long-term dependencies, dynamic variables such as temperature, humidity, and survival of probiotics during the drying process could be processed, and the prediction of probiotic loss based on historical data could be provided with $R^2 > 0.96$.

Probiotics are also exposed to serious acidic stresses in the low pH environment of the stomach. Prebiotics stand out as components that cannot be digested in the digestive system and greatly help maintain the viability of probiotics by feeding them in the large intestine. Inulin is also a fructan with prebiotic properties. In a study conducted by Chutrtong et al. [188], ML was used to evaluate the effects of changing inulin levels on the survival of probiotic microorganisms. It was stated that three different ML algorithms were used to examine the relationship between inulin levels and probiotic survival rates. The first of these was support vector machines (SVMs), in which the algorithm used decision boundaries to classify data and determined the boundaries that would be best for the discrimination of the data. The second was Naive Bayes, which calculated the effect of each feature on class prediction with Bayes' theorem. It was effective in small datasets. The third was the Decision Tree algorithm, which tried to predict the correct class at each decision point. A Confusion Matrix was used to estimate the accuracy of the model. This matrix showed the number of correct and incorrect predictions and was evaluated with measures such as precision and recall, measuring the success of the model.

One of the recently developed strategies against the low pH and bile salt stresses that probiotics are exposed to during digestion is to support them with coating technologies. However, the selection of excipients in the development of formulations is of great importance in terms of affecting the viability and colonization ability of probiotics. In response to this problem, the effects of excipients on probiotic growth have been tried to be analyzed and predicted using active ML [131]. Active ML can advantageously work with small datasets. After creating a model with known data, it can make predictions for unknown data and indicate how confident it is about which predictions. After testing less reliable data, the model is retrained to strengthen its overall accuracy of the model [189]. McCobrey et al. [14] also used six excipient–probiotic interaction datasets to predict the effects of 111 excipients on *Lacticaseibacillus paracasei*. As a result, the average accuracy rate of the model was 67.7%, while after experimental tests, the predictions were shown to be 75% accurate. Besides viability, another important technological feature of probiotics is their functionality. The various biologically active metabolites produced by starter LAB

used commercially in the production of fermented foods could determine the functional nutritional properties of the final product [190]. The utilization of probiotics and prebiotics to form a synbiotic formula is such good example to improve the nutritional as well as functional properties of fermented products [191]. The emergence of significant functional differences, even with small changes in the genome of LAB, makes it difficult to determine the optimal starter combinations, considering the huge diversity in their genomes [192]. In this regard, an AI-based method developed can be a useful tool in determining optimal combinations. For instance, a semi-supervised learning approach was used in a study aimed at developing an AI-based model that could predict the interactions of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria in milk fermentation. According to this approach, after analyzing the genome data of 362 bacterial isolates, half of which were *Lactobacillus bulgaricus* and the other half were *Streptococcus thermophilus*, the model was designed with a prediction system consisting of two components: the co-clustering model and the LapRLS model. While the co-clustering model predicted interactions based on genetic data in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, LapRLS worked using the Laplacian regularized least squares model based on K-mer analysis and gene compositions of the bacteria. The most accurate predictions were obtained by combining the outputs of these two models. When milk fermentation experiments were performed to see the accuracy of the model, it was seen that 17 of the 20 randomly selected bacterial combinations were predicted correctly, i.e., the success rate was 85% [192]. All these findings provided a basis for the utilization of the AI tools to improve the technological capacity of probiotics, and more studies are required to unveil new tools and models that could learn and improve the technological performances of probiotic strains.

6. Boosting Approach with AI to Increase the Capacity of Probiotics

Recent interests in probiotic science include increasing the potential therapeutic functions of probiotics by improving the current knowledge on probiotic mechanisms of action, targeting specific metabolites related to certain health functions, and developing personalized perceptions for individuals [117]. In both scenarios, AI approaches, technology, and tools have emerged and gained special interest in improving probiotic efficacy. In addition, key factors such as the time-consuming nature of traditional methods used to identify probiotic microorganisms, and the need for specialized and intensive resources, have led to the introduction of artificial intelligence applications in this field [123,157,158]. In a study on this subject, ProbML, an ML-based approach to identify probiotic organisms from whole genome sequences of prokaryotes, was introduced. Among the tested ML algorithms, XGBoost models were reported to show superior performance, achieving 95.45% accuracy on the independent test dataset. Using this approach, a robust, optimized, and highly reliable model was developed to accurately predict probiotic genomes from whole genome sequences of prokaryotic organisms by learning to recognize patterns and genetic markers indicative of probiotic properties [158]. In particular, AI and DL approaches have become some of the systematic methodologies to address the functional roles of probiotics for the gut microbiome [14,86,104,150]. A deep understanding of the microbiome could be applied using machine learning, which targets the monitoring, phenotyping, and classification of microbial species and consideration of the interactions of microbes with gut metabolites [123,193]. In a recent study, Westfall et al. [104], utilized an ML algorithm to explore the specific activity of certain probiotics to form metabolites with therapeutic functions, and this algorithm was suggested to be used for the generation of novel probiotic formulations. Another AI model developed recently to increase the efficiency of probiotics was DeepMicro, which could be trained on the outputs of microbiome data to predict the gut microbiome associations with certain diseases [194], and these outcomes might be shaped

for combating the associated diseases. As new terminologies occur in the context of probiotics such as paraprobiotics or postbiotics determining mainly the cell surface elements or intra/extracellular components of probiotics, respectively, that have positive effects on the health status of the host via different mechanisms, AI tools might pave the way to interact these metabolites with certain mechanisms as discussed previously [117,195]. A predictive approach to certain metabolites, whether attached to the cell surface of probiotics or present in the cytoplasm or extracellular environment, using AI tools, might form a platform to match these individual metabolites with certain modes of action of probiotics. In terms of predictive AI models, a recent study used ML models to select the superior probiotic strain among a number of important LAB strains based on their antimicrobial capacity [43]. Another AI-driven strategy to trigger the probiotic capacity is using the molecular docking approaches, and two recent examples in a food formulation [196] as well as the nanosynbiotics development process [197] provided the basis of these approaches as a promising methodology. In the latter study, the bioavailability and stability of the nanosynbiotics within the GI absorption conditions were predicted by AI-based models [197]. A study conducted to predict how excipients used to deliver probiotics live to the distal intestine affect the intestinal proliferation of an important probiotic, *Lactobacillus paracasei*, used a novel machine learning technique known as active ML. Starting with a labeled dataset consisting of only six bacteria–excipient interactions, active ML was able to predict the effects of 111 additional excipients using uncertainty sampling. It has been stated that the model can be used to enable superior probiotic delivery to maximize proliferation in vivo [14]. Another important issue that should be emphasized is the progress of the in silico methodologies such as metagenome sequencing, genome engineering, and others, which led the probiotic research to enter a new phase, determined as precision probiotics and next-generation probiotics. Similar to conventional probiotic research, AI tools could provide a powerful basis to understand the functionality of next-generation probiotics as well as engineered probiotics targeting specific health issues and providing potential therapeutic assistance [145]. This understanding should also consider the safety perspective of view for NGPs as the safety of these new candidate probiotics is still one of the main concerns that will determine their utilization [79]. The development of tools like iProbiotics, an ML-based platform for the prediction of the probiotic potential of distinct strains [150], will help us to unveil the health contribution of NGPs as well as their safety issues. Similar to precision probiotics, personalized probiotics combined with distinct prebiotics have become one of the key issues that will potentially play roles in shaping the future of probiotic research, in which AI tools could be critically harnessed [198]. A recent study provided such a good example of an AI-based personalized diet where microbiome modulation to improve the IBS-related symptoms in patients was directed by AI-based models [12]. AI tools could form a better perspective for personalized solutions following the probiotic applications, as the unique models could be applied to explore the interaction of gut microbiome and diet with their contribution to health and disease conditions. ML strategies could also be more informative to unveil certain interactions for probiotic functional food production processes. Liao et al. [199] demonstrated the interactions of three probiotic strains during fermented blueberry juice production with each other, together with the metabolites, using an ML optimization methodology, and using this methodology, the highest levels of the potential therapeutic metabolites in the juice were produced. Introduction of established probiotics to the market is also another side of the probiotic studies, and a recent study clearly demonstrated the utilization of the machine learning approach to characterize the consumer demands and their expectations, as well as trends in the probiotic market [200]. This AI approach clearly represented the interactions of the consumer preferences, including regulations and the industrial perspective of the companies, to allow

the reliable market conditions [200]. Overall, effective utilization of AI tools could pave the way for a better understanding of probiotic functionality as conventional or NGPs, via exploring the interactions of probiotics with the host as well as the production of certain therapeutic metabolites. More research on AI tools is clearly required to implement this indispensable methodology to boost the functionality of probiotics.

7. Conclusions and Research Outlook

Understanding the functionality of probiotics is a highly complex process since it is affected by distinct factors, including the exact role of proposed probiotics at the strain level, interactions of probiotics with the host's microbiome mainly within the gut, production of specific metabolites during host–probiotic interactions affecting host physiology as well as immune capacity. In this context, it is anticipated that technological developments such as artificial intelligence and machine learning will be important tools for the development of personalized synbiotics that can change according to the needs and goals of the individual in the future. At the same time, Al can be considered for the management of chronic diseases (e.g., IBS, obesity, diabetes) by generating personalized probiotic formulations according to the genetic structure, dietary habits, and microbiome structure of individuals. In addition, modeling the mechanisms of action of probiotics with artificial intelligence can be used to simulate how a probiotic microorganism acts in the human body, which can save time and cost in preclinical research. Modeling the complex interactions between nutrients and probiotics will also enable the development of useful approaches that can predict which diet and which probiotics provide more effective results. By combining AI technologies with other advanced genomic features, future models will be able to better explain the complex and overlapping characteristics of probiotic and non-probiotic bacterial genomes. For more in-depth analysis of microbiome data, machine learning, and deep learning techniques, in particular, can be used to unravel more complex relationships of microbiome data. In this way, interactions between different bacterial species can be better understood and microbiome profiles associated with certain health conditions can be identified. Additionally, AI models are important for understanding how probiotics can manipulate the composition of the gut microbiota and provide a controlled environment to test metabolite production. Evaluating these complex interactions with a machine-learning algorithm may allow for the elucidation of synergistic interactions. Furthermore, potential new synergisms that may not have been tested due to the inherent limitations of biological models can be predicted with this modeling, new therapeutic paradigms utilizing the human gut microbiota may also be optimized for metabolite production since the identification and microbiome analysis process currently rely on high-throughput omics technologies such as genomics, metabolomics, and proteomics. However, owing to the varying complexity degrees of microbiome-based big data, accessibility of a significant portion of the data has become extremely difficult and also requires a considerable amount of time. In this perspective, ML models can be more effective for different purposes, such as microbial phenotyping, microbial trait selection, and trait classification using molecular profiling data of microbes. Moreover, AI-enabled bioinformatics tools in the discovery of new probiotic strains can be used to determine previously unidentified beneficial microorganisms from metagenomic data, since these microorganisms may then be isolated in the laboratory and form the basis of new probiotic products. Finally, artificial intelligence is a promising tool for designing genetically engineered probiotics for specific purposes. The recognition of AI tools for probiotic research is crucial, but it is also critical to widen the current knowledge and to ensure the standardization of AI-driven technologies for different aspects, including personalized probiotic interventions as well as ethical and regulatory perspectives. It is obvious that more studies are required to further expand our knowledge of AI integration

with probiotic research and to deepen our understanding of utilizing AI tools to boost probiotic functionality.

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References

1. Zendeboodi, F.; Khorshidian, N.; Mortazavian, A.M.; da Cruz, A.G. Probiotic: Conceptualization from a new approach. *Curr. Opin. Food Sci.* **2020**, *32*, 103–123. [CrossRef]
2. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [CrossRef] [PubMed]
3. Plaza-Díaz, J.; Solís-Urra, P.; Rodríguez-Rodríguez, F.; Olivares-Arancibia, J.; Navarro-Oliveros, M.; Abadía-Molina, F.; Álvarez-Mercado, A.I. The gut barrier, intestinal microbiota, and liver disease: Molecular mechanisms and strategies to manage. *Int. J. Mol. Sci.* **2020**, *21*, 8351. [CrossRef] [PubMed]
4. Al-Fakhrany, O.M.; Elekhnawy, E. Next-generation probiotics: The upcoming biotherapeutics. *Mol. Biol. Rep.* **2024**, *51*, 505. [CrossRef]
5. O'Toole, P.W.; Marchesi, J.R.; Hill, C. Next-generation probiotics: The spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.* **2017**, *2*, 17057. [CrossRef]
6. Sharma, V.; Mobeen, F.; Prakash, T. Exploration of survival traits, probiotic determinants, host interactions, and functional evolution of bifidobacterial genomes using comparative genomics. *Genes* **2018**, *9*, 477. [CrossRef]
7. Dianawati, D.; Mishra, V.; Shah, N.P. Survival of microencapsulated probiotic bacteria after processing and during storage: A review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1685–1716. [CrossRef]
8. Zhang, L.; Ma, H.; Kulyar, M.F.-e.-A.; Pan, H.; Li, K.; Li, A.; Mo, Q.; Wang, Y.; Dong, H.; Bao, Y.; et al. Complete genome analysis of *Lactobacillus fermentum* YLF016 and its probiotic characteristics. *Microb. Pathog.* **2022**, *162*, 105212. [CrossRef]
9. Josephs-Spaulding, J.; Rajput, A.; Hefner, Y.; Szubin, R.; Balasubramanian, A.; Li, G.; Zielinski Daniel, C.; Jahn, L.; Sommer, M.; Phaneuf, P.; et al. Reconstructing the transcriptional regulatory network of probiotic *L. reuteri* is enabled by transcriptomics and machine learning. *mSystems* **2024**, *9*, e01257-23. [CrossRef]
10. Zhang, H.; Zhang, S.; Chen, L.; Xu, R.; Zhu, J. LC-HRMS-based metabolomics and lipidomics analyses of a novel probiotic *Akkermansia muciniphila* in response to different nutritional stimulations. *J. Microbiol. Methods* **2024**, *223*, 106975. [CrossRef]
11. Zhang, X.; Zheng, Y.; Liu, Z.; Su, M.; Wu, Z.; Zhang, H.; Zhang, C.; Xu, X. Insights into characteristic metabolites and potential bioactive peptides profiles of fresh cheese fermented with three novel probiotics based metabolomics and peptidomics. *Food Chem. X* **2024**, *21*, 101147. [CrossRef] [PubMed]
12. Karakan, T.; Gundogdu, A.; Alagözlü, H.; Ekmen, N.; Ozgul, S.; Tunali, V.; Hora, M.; Beyazgul, D.; Nalbantoglu, O.U. Artificial intelligence-based personalized diet: A pilot clinical study for irritable bowel syndrome. *Gut Microbes* **2022**, *14*, 2138672. [CrossRef] [PubMed]
13. Kwoji, I.D.; Aiyegoro, O.A.; Okpeku, M.; Adeleke, M.A. 'Multi-omics' data integration: Applications in probiotics studies. *NPJ Sci. Food* **2023**, *7*, 25. [CrossRef]
14. McCoubrey, L.E.; Seegobin, N.; Elbadawi, M.; Hu, Y.; Orlu, M.; Gaisford, S.; Basit, A.W. Active machine learning for formulation of precision probiotics. *Int. J. Pharm.* **2022**, *616*, 121568. [CrossRef]
15. Dossou, B.F.; Liu, D.; Ji, X.; Jain, M.; van der Sloot, A.M.; Palou, R.; Tyers, M.; Bengio, Y. Graph-based active machine learning method for diverse and novel antimicrobial peptides generation and selection. *arXiv* **2022**, arXiv:2209.13518.
16. Wu, S.; Feng, J.; Liu, C.; Wu, H.; Qiu, Z.; Ge, J.; Sun, S.; Hong, X.; Li, Y.; Wang, X. Machine learning aided construction of the quorum sensing communication network for human gut microbiota. *Nat. Commun.* **2022**, *13*, 3079. [CrossRef]
17. Pensupa, N.; Treebuppachartsakul, T.; Pechprasarn, S. Machine learning models using data mining for biomass production from *Yarrowia lipolytica* fermentation. *Fermentation* **2023**, *9*, 239. [CrossRef]
18. Florea, A.; Sipos, A.; Stoisor, M.-C. Applying AI tools for modeling, predicting and managing the white wine fermentation process. *Fermentation* **2022**, *8*, 137. [CrossRef]

19. Bertorello, S.; Cei, F.; Fink, D.; Niccolai, E.; Amedei, A. The future exploring of gut microbiome-immunity interactions: From in vivo/vitro models to in silico innovations. *Microorganisms* **2024**, *12*, 1828. [CrossRef]
20. Sadeghirashed, S.; Kazemi, F.; Taheri, S.; Ebrahimi, M.T.; Arasteh, J. A novel probiotic strain exerts therapeutic effects on mouse model of multiple sclerosis by altering the expression of inflammasome and IDO genes and modulation of T helper cytokine profile. *Metab. Brain Dis.* **2022**, *37*, 197–207. [CrossRef]
21. Han, X.; Liu, Q.; Li, Y.; Zhang, M.; Liu, K.; Kwok, L.-Y.; Zhang, H.; Zhang, W. Synergizing artificial intelligence and probiotics: A comprehensive review of emerging applications in health promotion and industrial innovation. *Trends Food Sci. Technol.* **2025**, *159*, 104938. [CrossRef]
22. Vulpoi, R.A.; Luca, M.; Ciobanu, A.; Olteanu, A.; Bărboi, O.; Iov, D.-E.; Nichita, L.; Ciortescu, I.; Cijevschi Prelipcean, C.; Ștefănescu, G.; et al. The potential use of artificial intelligence in irritable bowel syndrome management. *Diagnostics* **2023**, *13*, 3336. [CrossRef]
23. Karim, M.R.; Morshed, M.N.; Iqbal, S.; Mohammad, S.; Mathiyalagan, R.; Yang, D.C.; Kim, Y.J.; Song, J.H.; Yang, D.U. A network pharmacology and molecular-docking-based approach to identify the probable targets of short-chain fatty-acid-producing microbial metabolites against kidney cancer and inflammation. *Biomolecules* **2023**, *13*, 1678. [CrossRef]
24. Gatineau, G.; Shevroja, E.; Vendrami, C.; Gonzalez-Rodriguez, E.; Leslie, W.D.; Lamy, O.; Hans, D. Development and reporting of artificial intelligence in osteoporosis management. *J. Bone Miner. Res.* **2024**, *39*, 1553–1573. [CrossRef]
25. Li, Y.; Liu, X.; Cao, Z.; Wu, J. Probiotic supplements benefit psoriasis therapy rather than affecting disease risk: Evidence from NHANES machine-learning and meta-analysis study. *Probiotics Antimicrob. Proteins* **2024**, *16*, 1–15. [CrossRef]
26. Rugji, J.; Erol, Z.; Taşçı, F.; Musa, L.; Hamadani, A.; Gündemir, M.G.; Karalliu, E.; Siddiqui, S.A. Utilization of AI—Reshaping the future of food safety, agriculture and food security—A critical review. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 1–45. [CrossRef]
27. Bilal, H.; Khan, M.N.; Khan, S.; Shafiq, M.; Fang, W.; Khan, R.U.; Rahman, M.U.; Li, X.; Lv, Q.-L.; Xu, B. The role of artificial intelligence and machine learning in predicting and combating antimicrobial resistance. *Comput. Struct. Biotechnol. J.* **2025**, *27*, 423–439. [CrossRef]
28. Jeong, J.; Kim, S. Application of machine learning for quantitative analysis of industrial fermentation using image processing. *Food Sci. Biotechnol.* **2025**, *34*, 373–381. [CrossRef]
29. Zhang, X.; Elliot, M.A. Unlocking the trove of metabolic treasures: Activating silent biosynthetic gene clusters in bacteria and fungi. *Curr. Opin. Microbiol.* **2019**, *51*, 9–15. [CrossRef]
30. Yue, L.; Song, L.; Zhu, S.; Fu, X.; Li, X.; He, C.; Li, J. Machine learning assisted rational design of antimicrobial peptides based on human endogenous proteins and their applications for cosmetic preservative system optimization. *Sci. Rep.* **2024**, *14*, 947. [CrossRef]
31. Liu, Y.; Yu, W.; Wang, Q.; Cao, Z.; Li, J. Artificially engineered bacteria to treat gastrointestinal disease and cancer. *Drug Discov. Today* **2023**, *28*, 103667. [CrossRef] [PubMed]
32. Ding, J.; Wang, B.; Liu, Q.; Hou, W.; Cai, J.; Lu, C. Modeling and optimization of sporulation by *Bacillus licheniformis* BF-002 based on dynamics and recurrent neural networks. *Bioresour. Technol.* **2024**, *398*, 130534. [CrossRef] [PubMed]
33. Hameed, A.; Condò, C.; Tauseef, I.; Idrees, M.; Ghazanfar, S.; Farid, A.; Muzammal, M.; Al Mohaini, M.; Alsalman, A.J.; Al Hawaj, M.A.; et al. Isolation and characterization of a cholesterol-lowering bacteria from *Bubalus bubalis* raw milk. *Fermentation* **2022**, *8*, 163. [CrossRef]
34. Golnari, M.; Bahrami, N.; Milanian, Z.; Rabbani Khorasgani, M.; Asadollahi, M.A.; Shafiei, R.; Fatemi, S.S.-A. Isolation and characterization of novel Bacillus strains with superior probiotic potential: Comparative analysis and safety evaluation. *Sci. Rep.* **2024**, *14*, 1457. [CrossRef]
35. dos Santos Leandro, E.; Ginani, V.C.; de Alencar, E.R.; Pereira, O.G.; Rose, E.C.P.; do Vale, H.M.M.; Pratesi, R.; Hecht, M.M.; Cavalcanti, M.H.; Tavares, C.S.O. Isolation, identification, and screening of lactic acid bacteria with probiotic potential in silage of different species of forage plants, cocoa beans, and artisanal salami. *Probiotics Antimicrob. Proteins* **2021**, *13*, 173–186. [CrossRef]
36. Amenu, D.; Bacha, K. Probiotic potential and safety analysis of lactic acid bacteria isolated from Ethiopian traditional fermented foods and beverages. *Ann. Microbiol.* **2023**, *73*, 37. [CrossRef]
37. Wu, H.; Shum, T.-F.; Chiou, J. Characterization of the probiotic potential of lactic acid bacteria isolated from kimchi, yogurt, and baby feces in Hong Kong and their performance in soymilk fermentation. *Microorganisms* **2021**, *9*, 2544. [CrossRef]
38. Bihola, A.; Jana, A.H.; Parmar, S.C.; Adil, S. Feasibility study of utilizing Saccharomyces boulardii as an adjunct culture in Mozzarella type cheese and its quality characterization. *Discov. Food* **2024**, *4*, 105. [CrossRef]
39. Vergara Alvarez, S.C.; Pendón, M.D.; Bengoa, A.A.; Leiva Alaniz, M.J.; Maturano, Y.P.; Garrote, G.L. Probiotic potential of yeasts isolated from fermented beverages: Assessment of antagonistic strategies against *Salmonella enterica* serovar enteritidis. *J. Fungi* **2024**, *10*, 878. [CrossRef]
40. da Silva, M.N.; Tagliapietra, B.L.; do Amaral Flores, V.; dos Santos Richards, N.S.P. In vitro test to evaluate survival in the gastrointestinal tract of commercial probiotics. *Curr. Res. Food Sci.* **2021**, *4*, 320–325. [CrossRef]

41. Abdi, A.; Gatri, E.; Guilbaud, J.; Bouallagui, H.; Fadhlaoui, K.; Garrait, G.; Ayed, L.; Gatri, E.; Guilbaud, J.; Bouallagui, H.; et al. Co-cultivation of potential probiotic strains isolated from water kefir for fermented green tea beverage. *Fermentation* **2025**, *11*, 169. [CrossRef]
42. Shehata, M.G.; Masry, S.H.; Abd El-Aziz, N.M.; Ridouane, F.L.; Mirza, S.B.; El-Sohaimy, S.A. Probiotic potential of lactic acid bacteria isolated from honeybees stomach: Functional and technological insights. *Ann. Agric. Sci.* **2024**, *69*, 11–18. [CrossRef]
43. Sadeghi, M.; Panahi, B.; Mazlumi, A.; Hejazi, M.A.; Komi, D.E.A.; Nami, Y. Screening of potential probiotic lactic acid bacteria with antimicrobial properties and selection of superior bacteria for application as biocontrol using machine learning models. *LWT* **2022**, *162*, 113471. [CrossRef]
44. Xing, T.-l.; Bian, X.; Ma, C.-m.; Yang, Y.; Liu, X.-f.; Wang, Y.; Fan, J.; Zhang, N. In vitro evaluation of probiotic properties of Lactobacillus acidophilus AD125 and antagonism against Escherichia coli O157: H7 adhesion to Caco-2 cell. *Food Funct.* **2023**, *14*, 2472–2480. [CrossRef]
45. Chantanawilas, P.; Pahumunto, N.; Teanpaisan, R. Aggregation and adhesion ability of various probiotic strains and Candida species: An in vitro study. *J. Dent. Sci.* **2024**, *19*, 2163–2171. [CrossRef]
46. Wang, Y.; Liang, Q.; Lu, B.; Shen, H.; Liu, S.; Shi, Y.; Leptihn, S.; Li, H.; Wei, J.; Liu, C. Whole-genome analysis of probiotic product isolates reveals the presence of genes related to antimicrobial resistance, virulence factors, and toxic metabolites, posing potential health risks. *BMC Genom.* **2021**, *22*, 210. [CrossRef]
47. Kim, Y.; Choi, S.-I.; Jeong, Y.; Kang, C.-H. Evaluation of safety and probiotic potential of Enterococcus faecalis MG5206 and Enterococcus faecium MG5232 isolated from Kimchi, a Korean fermented cabbage. *Microorganisms* **2022**, *10*, 2070. [CrossRef]
48. Vinayamohan, P.G.; Viju, L.S.; Joseph, D.; Venkitanarayanan, K. Fermented foods as a potential vehicle of antimicrobial-resistant bacteria and genes. *Fermentation* **2023**, *9*, 688. [CrossRef]
49. Gizachew, S.; Van Beeck, W.; Spacova, I.; Dekeukeleire, M.; Alemu, A.; Woldemedhin, W.M.; Mariam, S.H.; Lebeer, S.; Engidawork, E. Antibacterial and Immunostimulatory activity of potential probiotic lactic acid bacteria isolated from Ethiopian fermented dairy products. *Fermentation* **2023**, *9*, 258. [CrossRef]
50. Poimenidou, S.V.; Skarveli, A.; Saxami, G.; Mitsou, E.K.; Kotsou, M.; Kyriacou, A. Inhibition of Listeria monocytogenes growth, adherence and invasion in Caco-2 cells by potential probiotic lactic acid bacteria isolated from Fecal samples of healthy neonates. *Microorganisms* **2023**, *11*, 363. [CrossRef]
51. Barzegar, H.; Alizadeh Behbahani, B.; Falah, F. Safety, probiotic properties, antimicrobial activity, and technological performance of Lactobacillus strains isolated from Iranian raw milk cheeses. *Food Sci. Nutr.* **2021**, *9*, 4094–4107. [CrossRef] [PubMed]
52. Ajibade, B.O.; Ajayeoba, T.A.; Sabiu, S.; Moiseenko, K.V.; Mbona, S.V.; Cason, E.D.; Fedorova, T.V.; Ijabadeniyi, O.A. Unveiling the microbial symphony of amasi: A targeted metagenomic 16S rRNA, ITS, and metabolites insights using bovine and caprine milk. *Fermentation* **2025**, *11*, 6. [CrossRef]
53. Chang, H.M.; Foo, H.L.; Loh, T.C.; Lim, E.T.C.; Abdul Mutalib, N.E. Comparative studies of inhibitory and antioxidant activities, and organic acids compositions of postbiotics produced by probiotic Lactiplantibacillus plantarum strains isolated from Malaysian foods. *Front. Vet. Sci.* **2021**, *7*, 602280. [CrossRef] [PubMed]
54. Ali, A.H.; Bamigbade, G.; Tarique, M.; Esposito, G.; Obaid, R.; Abu-Jdayil, B.; Ayyash, M. Physicochemical, rheological, and bioactive properties of exopolysaccharide produced by a potential probiotic Enterococcus faecalis 84B. *Int. J. Biol. Macromol.* **2023**, *240*, 124425. [CrossRef]
55. Cho, H.; Jo, M.; Oh, H.; Lee, Y.; Park, Y. Synergistic antidepressant-like effect of n-3 polyunsaturated fatty acids and probiotics through the brain-gut axis in rats exposed to chronic mild stress. *J. Nutr. Biochem.* **2023**, *116*, 109326. [CrossRef]
56. Noda, M.; Danshiitsoodol, N.; Sakaguchi, T.; Kanno, K.; Sugiyama, M. Exopolysaccharide produced by plant-derived Lactobacillus plantarum SN35N exhibits antiviral activity. *Biol. Pharm. Bull.* **2021**, *44*, 1886–1890. [CrossRef]
57. Nehal, F.; Sahnoun, M.; Smaoui, S.; Jaouadi, B.; Bejar, S.; Mohammed, S. Characterization, high production and antimicrobial activity of exopolysaccharides from Lactococcus lactis F-mou. *Microb. Pathog.* **2019**, *132*, 10–19. [CrossRef]
58. Sugimura, N.; Li, Q.; Chu, E.S.H.; Lau, H.C.H.; Fong, W.; Liu, W.; Liang, C.; Nakatsu, G.; Su, A.C.Y.; Coker, O.O. Lactobacillus gallinarum modulates the gut microbiota and produces anti-cancer metabolites to protect against colorectal tumourigenesis. *Gut* **2022**, *71*, 2011–2021. [CrossRef]
59. Afreen, A.; Ahmed, Z.; Khalid, N.; Ferheen, I.; Ahmed, I. Optimization and cholesterol-lowering activity of exopolysaccharide from Lactiplantibacillus paraplantarum NCCP 962. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 1189–1204. [CrossRef]
60. Park, J.M.; Lee, S.C.; Ham, C.; Kim, Y.W. Effect of probiotic supplementation on gastrointestinal motility, inflammation, motor, non-motor symptoms and mental health in Parkinson's disease: A meta-analysis of randomized controlled trials. *Gut Pathog.* **2023**, *15*, 9. [CrossRef]
61. Juntarachot, N.; Sunpaweravong, S.; Kaewdech, A.; Wongsuwanlert, M.; Ruangsri, P.; Pahumunto, N.; Teanpaisan, R. Characterization of adhesion, anti-adhesion, co-aggregation, and hydrophobicity of Helicobacter pylori and probiotic strains. *J. Taibah Univ. Med. Sci.* **2023**, *18*, 1048–1054. [CrossRef] [PubMed]

62. Kumar, R.; Bansal, P.; Singh, J.; Dhanda, S. Purification, partial structural characterization and health benefits of exopolysaccharides from potential probiotic Pediococcus acidilactici NCDC 252. *Process Biochem.* **2020**, *99*, 79–86. [CrossRef]
63. Gawande, K.; Kolhekar, M.; Kumari, M.; Kapila, S.; Sharma, P.; Ali, S.A.; Behare, P.V. Lactic acid bacteria based purified exopolysaccharide showed viscofying and hypercholesterolemic capabilites. *Food Hydrocoll. Health* **2021**, *1*, 100042. [CrossRef]
64. Nurkanto, A.; Fanani, A.; Nurcahyanto, D.A.; Mamangkey, J.; Marissa, S.; Pasaribu, K.M.; Afiati, F.; Agustini, N.W.S.; Ahmad, R.Z.; Taufik, M. Anti-hypercholesterolemia properties of exopolysaccharide from Lactiplantibacillus plantarum MI01: Computational and in-vivo approaches. *Case Stud. Chem. Environ. Eng.* **2025**, *11*, 101146.
65. Khalil, M.A.; Sonbol, F.I.; Al-Madboly, L.A.; Aboshady, T.A.; Alqurashi, A.S.; Ali, S.S. Exploring the therapeutic potentials of exopolysaccharides derived from lactic acid bacteria and bifidobacteria: Antioxidant, antitumor, and periodontal regeneration. *Front. Microbiol.* **2022**, *13*, 803688. [CrossRef]
66. Sasikumar, K.; Vaikkath, D.K.; Devendra, L.; Nampoothiri, K.M. An exopolysaccharide (EPS) from a Lactobacillus plantarum BR2 with potential benefits for making functional foods. *Bioresour. Technol.* **2017**, *241*, 1152–1156. [CrossRef]
67. Khromova, N.Y.; Epishkina, J.M.; Karetkin, B.A.; Khabibulina, N.V.; Beloded, A.V.; Shakir, I.V.; Panfilov, V.I. The combination of in vitro assessment of stress tolerance ability, autoaggregation, and vitamin B-producing ability for new probiotic strain introduction. *Microorganisms* **2022**, *10*, 470. [CrossRef]
68. Kim, J.-Y.; Choi, E.-J.; Lee, J.-H.; Yoo, M.-S.; Heo, K.; Shim, J.-J.; Lee, J.-L. Probiotic potential of a novel vitamin B2-overproducing Lactobacillus plantarum strain, HY7715, isolated from Kimchi. *Appl. Sci.* **2021**, *11*, 5765. [CrossRef]
69. Carrizo, S.L.; de LeBlanc, A.d.M.; LeBlanc, J.G.; Rollán, G.C. Quinoa pasta fermented with lactic acid bacteria prevents nutritional deficiencies in mice. *Food Res. Int.* **2020**, *127*, 108735. [CrossRef]
70. Yépez, A.; Russo, P.; Spano, G.; Khomenko, I.; Biasioli, F.; Capozzi, V.; Aznar, R. In situ riboflavin fortification of different kefir-like cereal-based beverages using selected Andean LAB strains. *Food Microbiol.* **2019**, *77*, 61–68. [CrossRef]
71. Thananimit, S.; Pahumunto, N.; Teanpaisan, R. Characterization of short chain fatty acids produced by selected potential probiotic lactobacillus strains. *Biomolecules* **2022**, *12*, 1829. [CrossRef]
72. Alan, Y.; Savcı, A.; Koçpınar, E.F.; Ertaş, M. Postbiotic metabolites, antioxidant and anticancer activities of probiotic Leuconostoc pseudomesenteroides strains in natural pickles. *Arch. Microbiol.* **2022**, *204*, 571. [CrossRef]
73. Jeong, S.; Kim, Y.; Park, S.; Lee, D.; Lee, J.; Hlaing, S.P.; Yoo, J.-W.; Rhee, S.H.; Im, E. Lactobacillus plantarum metabolites elicit anticancer effects by inhibiting autophagy-related responses. *Molecules* **2023**, *28*, 1890. [CrossRef]
74. Abdel-Nasser, A.; Hathout, A.S.; Badr, A.N.; Barakat, O.S.; Fathy, H.M. Extraction and characterization of bioactive secondary metabolites from lactic acid bacteria and evaluating their antifungal and antiaflatoxigenic activity. *Biotechnol. Rep.* **2023**, *38*, e00799. [CrossRef]
75. Sentürk, M.; Ercan, F.; Yalcin, S. The secondary metabolites produced by Lactobacillus plantarum downregulate BCL-2 and BUFFY genes on breast cancer cell line and model organism Drosophila melanogaster: Molecular docking approach. *Cancer Chemother. Pharmacol.* **2020**, *85*, 33–45. [CrossRef]
76. Garcia-Gonzalez, N.; Bottacini, F.; Van Sinderen, D.; Gahan, C.G.; Corsetti, A. Comparative genomics of Lactiplantibacillus plantarum: Insights into probiotic markers in strains isolated from the human gastrointestinal tract and fermented foods. *Front. Microbiol.* **2022**, *13*, 854266. [CrossRef]
77. Lau, L.Y.J.; Quek, S.Y. Probiotics: Health benefits, food application, and colonization in the human gastrointestinal tract. *Food Bioeng.* **2024**, *3*, 41–64. [CrossRef]
78. Lalowski, P.; Zielińska, D. The most promising next-generation probiotic candidates—Impact on human health and potential application in food technology. *Fermentation* **2024**, *10*, 444. [CrossRef]
79. Saarela, M.H. Safety aspects of next generation probiotics. *Curr. Opin. Food Sci.* **2019**, *30*, 8–13. [CrossRef]
80. Cai, X.; Wen, J.-s.; Long, H.; Ren, W.; Zhang, X.; Huang, A.-y.; Xie, Z.-y. The probiotic effects, dose, and duration of lactic acid bacteria on disease resistance in *Litopenaeus vannamei*. *Aquac. Rep.* **2022**, *26*, 101299. [CrossRef]
81. Dai, X.; Shen, L. Advances and trends in omics technology development. *Front. Med.* **2022**, *9*, 911861. [CrossRef] [PubMed]
82. Akiyama, M. Multi-omics study for interpretation of genome-wide association study. *J. Hum. Genet.* **2021**, *66*, 3–10. [CrossRef]
83. Vahabi, N.; Michailidis, G. Unsupervised multi-omics data integration methods: A comprehensive review. *Front. Genet.* **2022**, *13*, 854752. [CrossRef]
84. Jan, R.; Hussain, A.; Assad, A.; Khurshid, S.; Macha, M.A. Chapter 10—Challenges with multi-omics data integration. In *Multi-Omics Technology in Human Health and Diseases*; Macha, M.A., Bhat, A.A., Masoodi, T.A., Eds.; Academic Press: Cambridge, MA, USA, 2025; pp. 223–242.
85. Gao, F.; Huang, K.; Xing, Y. Artificial intelligence in omics. *Genom. Proteom. Bioinform.* **2023**, *20*, 811–813. [CrossRef]
86. D'Urso, F.; Broccolo, F. Applications of artificial intelligence in microbiome analysis and probiotic interventions—An overview and perspective based on the current state of the art. *Appl. Sci.* **2024**, *14*, 8627. [CrossRef]
87. Xue, W.; Liu, C.; Liu, Y.; Ding, H.; An, C.; Zhang, S.; Ma, S.; Zhang, Q. Probiotic evaluation of *Lactiplantibacillus pentosus* 68-1, a rutin conversion strain isolated from jiangshui, by genomic analysis and tests in vitro. *Fermentation* **2024**, *10*, 87. [CrossRef]

88. Kankainen, M.; Paulin, L.; Tynkkynen, S.; von Ossowski, I.; Reunanen, J.; Partanen, P.; Satokari, R.; Vesterlund, S.; Hendrickx, A.P.A.; Lebeer, S.; et al. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human- mucus binding protein. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 17193–17198. [CrossRef]
89. Parimelzaghan, A.; Anbarasu, A.; Ramaiah, S. Gene network analysis of metallo beta lactamase family proteins indicates the role of gene partners in antibiotic resistance and reveals important drug targets. *J. Cell. Biochem.* **2016**, *117*, 1330–1339. [CrossRef]
90. Zhang, G.; He, M.; Xiao, L.; Jiao, Y.; Han, J.; Li, C.; Miller, M.J.; Zhang, L. Milk fat globule membrane protects *Bifidobacterium longum* ssp. *infantis* ATCC 15697 against bile stress by modifying global transcriptional responses. *J. Dairy Sci.* **2024**, *107*, 91–104. [CrossRef]
91. Peng, Y.; Chen, R.; Zhang, Z.; Jin, R.; Xie, T.; Liu, X.; Chai, J.; Howe, S.; Zhao, J.; Li, Y. Metagenomic and meta-transcriptomic analysis reveal the colonization and expression profile of probiotic strains in humans and animals. *Fermentation* **2023**, *9*, 417. [CrossRef]
92. Wang, J.; Zhang, J.; Liu, W.; Zhang, H.; Sun, Z. Metagenomic and metatranscriptomic profiling of Lactobacillus casei Zhang in the human gut. *NPJ Biofilms Microbiomes* **2021**, *7*, 55. [CrossRef] [PubMed]
93. Zhao, Y.; Wu, Z.; Miyao, S.; Zhang, W. Unraveling the flavor profile and microbial roles during industrial Sichuan radish paocai fermentation by molecular sensory science and metatranscriptomics. *Food Biosci.* **2022**, *48*, 101815. [CrossRef]
94. Bianchi, L.; Laghi, L.; Correani, V.; Schifano, E.; Landi, C.; Uccelletti, D.; Mattei, B. A combined proteomics, metabolomics and in vivo analysis approach for the characterization of probiotics in large-scale production. *Biomolecules* **2020**, *10*, 157. [CrossRef]
95. Siciliano, R.A.; Lippolis, R.; Mazzeo, M.F. Proteomics for the investigation of surface-exposed proteins in probiotics. *Front. Nutr.* **2019**, *6*, 52. [CrossRef]
96. Beck, H.C.; Madsen, S.M.; Glenting, J.; Petersen, J.; Israelsen, H.; Nørrelykke, M.R.; Antonsson, M.; Hansen, A.M. Proteomic analysis of cell surface-associated proteins from probiotic *Lactobacillus plantarum*. *FEMS Microbiol. Lett.* **2009**, *297*, 61–66. [CrossRef]
97. Klotz, C.; O'Flaherty, S.; Goh, Y.J.; Barrangou, R. Investigating the effect of growth phase on the surface-layer associated proteome of *Lactobacillus acidophilus* using quantitative proteomics. *Front. Microbiol.* **2017**, *8*, 2174. [CrossRef]
98. Senior, A.W.; Evans, R.; Jumper, J.; Kirkpatrick, J.; Sifre, L.; Green, T.; Qin, C.; Žídek, A.; Nelson, A.W.; Bridgland, A. Improved protein structure prediction using potentials from deep learning. *Nature* **2020**, *577*, 706–710. [CrossRef]
99. Sun, Z.; Ning, Z.; Figeys, D. The landscape and perspectives of the human gut metaproteomics. *Mol. Cell. Proteom.* **2024**, *23*, 100763. [CrossRef]
100. Kolmeder, C.A.; Salojärvi, J.; Ritari, J.; De Been, M.; Raes, J.; Falony, G.; Vieira-Silva, S.; Kekkonen, R.A.; Corthals, G.L.; Palva, A. Faecal metaproteomic analysis reveals a personalized and stable functional microbiome and limited effects of a probiotic intervention in adults. *PLoS ONE* **2016**, *11*, e0153294. [CrossRef]
101. Hendrickx, D.; An, R.; Boeren, S.; Mutte, S.; Wopereis, H.; Belzer, C.; PRESTO Study Team. Trackability of proteins from probiotic *Bifidobacterium* spp. in the gut using metaproteomics. *Benef. Microbes* **2023**, *14*, 269–280. [CrossRef]
102. Rueangsri, N.; Roytrakul, S.; Muangnoi, C.; Tongkhao, K.; Sae-Tan, S.; Treesuwan, K.; Sirivarasai, J. Metaproteomic analysis of fermented vegetable formulations with lactic acid bacteria: A comparative study from initial stage to 15 days of production. *Foods* **2025**, *14*, 1148. [CrossRef] [PubMed]
103. Sydor, S.; Dandyk, C.; Schwerdt, J.; Manka, P.; Benndorf, D.; Lehmann, T.; Schallert, K.; Wolf, M.; Reichl, U.; Canbay, A. Discovering biomarkers for non-alcoholic steatohepatitis patients with and without hepatocellular carcinoma using fecal metaproteomics. *Int. J. Mol. Sci.* **2022**, *23*, 8841. [CrossRef] [PubMed]
104. Westfall, S.; Carracci, F.; Estill, M.; Zhao, D.; Wu, Q.L.; Shen, L.; Simon, J.; Pasinetti, G.M. Optimization of probiotic therapeutics using machine learning in an artificial human gastrointestinal tract. *Sci. Rep.* **2021**, *11*, 1067. [CrossRef] [PubMed]
105. Nakamura, Y.; Suzuki, S.; Murakami, S.; Nishimoto, Y.; Higashi, K.; Watarai, N.; Umetsu, J.; Ishii, C.; Ito, Y.; Mori, Y.; et al. Integrated gut microbiome and metabolome analyses identified fecal biomarkers for bowel movement regulation by *Bifidobacterium longum* BB536 supplementation: A RCT. *Comput. Struct. Biotechnol. J.* **2022**, *20*, 5847–5858. [CrossRef]
106. Walczak-Skierska, J.; Ludwiczak, A.; Sibińska, E.; Pomastowski, P. Environmental influence on bacterial lipid composition: Insights from pathogenic and probiotic strains. *ACS Omega* **2024**, *9*, 37789–37801. [CrossRef]
107. Holčapek, M.; Liebisch, G.; Ekroos, K. Lipidomic analysis. *Anal. Chem.* **2018**, *90*, 4249–4257. [CrossRef]
108. Lin, W.-J.; Shen, P.-C.; Liu, H.-C.; Cho, Y.-C.; Hsu, M.-K.; Lin, I.-C.; Chen, F.-H.; Yang, J.-C.; Ma, W.-L.; Cheng, W.-C. LipidSig: A web-based tool for lipidomic data analysis. *Nucleic Acids Res.* **2021**, *49*, W336–W345. [CrossRef]
109. Schoug, Å.; Fischer, J.; Heipieper, H.J.; Schnürer, J.; Håkansson, S. Impact of fermentation pH and temperature on freeze-drying survival and membrane lipid composition of *Lactobacillus coryniformis* Si3. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 175–181. [CrossRef]
110. Schifano, E.; Cicalini, I.; Pieragostino, D.; Heipieper, H.J.; Del Boccio, P.; Uccelletti, D. In vitro and in vivo lipidomics as a tool for probiotics evaluation. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 8937–8948. [CrossRef]
111. Yang, F.; Zhu, W.; Edirisuriya, P.; Ai, Q.; Nie, K.; Ji, X.; Zhou, K. Characterization of metabolites and biomarkers for the probiotic effects of *Clostridium cochlearium* on high-fat diet-induced obese C57BL/6 mice. *Eur. J. Nutr.* **2022**, *61*, 2217–2229. [CrossRef]

112. Pokusaeva, K.; Fitzgerald, G.F.; van Sinderen, D. Carbohydrate metabolism in Bifidobacteria. *Genes Nutr.* **2011**, *6*, 285–306. [CrossRef] [PubMed]
113. Ströher, J.A.; Oliveira, W.D.C.; de Freitas, A.S.; Salazar, M.M.; da Silva, L.D.F.F.; Bresciani, L.; Flôres, S.H.; Malheiros, P.D.S. A global review of geographical diversity of kefir microbiome. *Fermentation* **2025**, *11*, 150. [CrossRef]
114. Durazzi, F.; Sala, C.; Castellani, G.; Manfreda, G.; Remondini, D.; De Cesare, A. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. *Sci. Rep.* **2021**, *11*, 3030. [CrossRef] [PubMed]
115. Carasi, P.; Racedo, S.M.; Jacquot, C.; Elie, A.M.; Serradell, M.L.; Urdaci, M.C. *Enterococcus durans* EP1 a promising anti-inflammatory probiotic able to stimulate siga and to increase *Faecalibacterium prausnitzii* abundance. *Front. Immunol.* **2017**, *8*, 88. [CrossRef]
116. Rozera, T.; Pasolli, E.; Segata, N.; Ianiro, G. Machine learning and artificial intelligence in the multi-omics approach to gut microbiota. *Gastroenterology* **2025**, *13*, 1–15. [CrossRef]
117. Das, A.; Behera, R.N.; Kapoor, A.; Ambatipudi, K. The potential of meta-proteomics and artificial intelligence to establish the next generation of probiotics for personalized healthcare. *J. Agric. Food Chem.* **2023**, *71*, 17528–17542. [CrossRef]
118. Feng, Y.; Soni, A.; Brightwell, G.; Reis, M.M.; Wang, Z.; Wang, J.; Wu, Q.; Ding, Y. The potential new microbial hazard monitoring tool in food safety: Integration of metabolomics and artificial intelligence. *Trends Food Sci. Technol.* **2024**, *149*, 104555. [CrossRef]
119. Wani, A.K.; Roy, P.; Kumar, V.; Mir, T.u.G. Metagenomics and artificial intelligence in the context of human health. *Infect. Genet. Evol.* **2022**, *100*, 105267. [CrossRef]
120. Shehata, H.R.; Hassane, B.; Newmaster, S.G. Real-time polymerase chain reaction methods for strain specific identification and enumeration of strain *Lacticaseibacillus paracasei* 8700:2. *Front. Microbiol.* **2023**, *13*, 1076631. [CrossRef]
121. Zhu, B.; Wang, X.; Li, L. Human gut microbiome: The second genome of human body. *Protein Cell* **2010**, *1*, 718–725. [CrossRef]
122. Goodswen, S.J.; Barratt, J.L.N.; Kennedy, P.J.; Kaufer, A.; Calarco, L.; Ellis, J.T. Machine learning and applications in microbiology. *FEMS Microbiol. Rev.* **2021**, *45*, fuab015. [CrossRef] [PubMed]
123. Marcos-Zambrano, L.J.; Karaduzovic-Hadziabdic, K.; Loncar Turukalo, T.; Przymus, P.; Trajkovik, V.; Aasmets, O.; Berland, M.; Gruca, A.; Hasic, J.; Hron, K. Applications of machine learning in human microbiome studies: A review on feature selection, biomarker identification, disease prediction and treatment. *Front. Microbiol.* **2021**, *12*, 634511. [CrossRef] [PubMed]
124. Davenport, T.; Kalakota, R. The potential for artificial intelligence in healthcare. *Future Healthc. J.* **2019**, *6*, 94–98. [CrossRef] [PubMed]
125. Al-Adham, I.S.; Agha, A.S.; Al-Akayleh, F.; Al-Remawi, M.; Jaber, N.; Al Manasur, M.; Collier, P.J. Prebiotics Beyond the Gut: Omics Insights, Artificial Intelligence, and Clinical Trials in Organ-Specific Applications. *Probiotics Antimicrob. Proteins* **2025**, 1–22. [CrossRef]
126. Krupitzer, C. Generative artificial intelligence in the agri-food value chain-overview, potential, and research challenges. *Front. Food Sci. Technol.* **2024**, *4*, 1473357. [CrossRef]
127. Badidi, E. Edge AI for early detection of chronic diseases and the spread of infectious diseases: Opportunities, challenges, and future directions. *Future Internet* **2023**, *15*, 370. [CrossRef]
128. Banerjee, N.; Chatterjee, K. Quantum AI in healthcare: Revolutionizing diagnosis, treatment and drug discovery. *Int. J. Sci. Res. Sci. Technol.* **2024**, *11*, 815–836. [CrossRef]
129. Abdelwahab, S.I.; Taha, M.M.E.; Jerah, A.A.; Farasani, A.; Abdullah, S.M.; Aljahdali, I.A.; Ibrahim, R.; Oraibi, O.; Oraibi, B.; Alfaifi, H.A.; et al. Artificial intelligence and microbiome research: Evolution of hotspots, research trends, and thematic-based narrative review. *Cell Mol. Biol.* **2024**, *70*, 182–192. [CrossRef]
130. Patil, A.; Singh, N.; Patwekar, M.; Patwekar, F.; Patil, A.; Gupta, J.K.; Elumalai, S.; Priya, N.S.; Sahithi, A. AI-driven insights into the microbiota: Figuring out the mysterious world of the gut. *Intell. Pharm.* **2025**, *3*, 46–52. [CrossRef]
131. McCoubrey, L.E.; Elbadawi, M.; Orlu, M.; Gaisford, S.; Basit, A.W. Harnessing machine learning for development of microbiome therapeutics. *Gut Microbes* **2021**, *13*, 1872323. [CrossRef]
132. Abavisani, M.; Foroushan, S.K.; Ebadpour, N.; Sahebkar, A. Deciphering the gut microbiome: The revolution of artificial intelligence in microbiota analysis and intervention. *Curr. Res. Biotechnol.* **2024**, *7*, 100211. [CrossRef]
133. Kobayashi, Y.; Uchida, T.; Inoue, T.; Iwasaki, Y.; Ito, R.; Saito, K.; Akiyama, H.; Tsuda, K.; Yoshida, K. Analysis of 50 years of health food research trends using natural language processing and generative AI. *Procedia Comput. Sci.* **2024**, *246*, 1875–1884. [CrossRef]
134. Myers, B.K.; Lamichhane, A.; Kvitko, B.H.; Dutta, B. Natural language processing-like deep learning aided in identification and validation of thiosulfinate tolerance clusters in diverse bacteria. *bioRxiv* **2024**. [CrossRef]
135. Danielsen, J.; Nordenfelt, P. Computer vision-based image analysis of bacteria. In *Bacterial Pathogenesis: Methods and Protocols*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 161–172.
136. Loddo, A.; Di Ruberto, C.; Armano, G.; Manconi, A. Automatic monitoring cheese ripeness using computer vision and artificial intelligence. *IEEE Access* **2022**, *10*, 122612–122626. [CrossRef]
137. Treloar, N.J.; Fedorec, A.J.; Ingalls, B.; Barnes, C.P. Deep reinforcement learning for the control of microbial co-cultures in bioreactors. *PLoS Comput. Biol.* **2020**, *16*, e1007783. [CrossRef]

138. Dhatterwal, J.S.; Kaswan, K.S.; Kumar, N. Robotic process automation in healthcare. In *Confluence of Artificial Intelligence and Robotic Process Automation*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 157–175.
139. Das, D.; Roy, A.D.; Gupta, S.D.; Roy, R.D. The use of generative artificial intelligence (AI) in teaching and assessment of postgraduate students in pathology and microbiology. *Indian J. Microbiol. Res.* **2024**, *3*, 140–146. [CrossRef]
140. Sionek, B.; Szydłowska, A.; Zielińska, D.; Neffe-Skocińska, K.; Kołożyn-Krajewska, D. Beneficial bacteria isolated from food in relation to the next generation of probiotics. *Microorganisms* **2023**, *11*, 1714. [CrossRef]
141. Zhai, Y.; Kim, M.; Fan, P.; Rajeev, S.; Kim, S.A.; Driver, J.D.; Galvão, K.N.; Boucher, C.; Jeong, K.C. Machine learning-enhanced assessment of potential probiotics from healthy calves for the treatment of neonatal calf diarrhea. *Front. Microbiol.* **2024**, *15*, 1507537. [CrossRef]
142. Yang, N.J.; Chiu, I.M. Bacterial signaling to the nervous system through toxins and metabolites. *J. Mol. Biol.* **2017**, *429*, 587–605. [CrossRef]
143. Takahashi, M.K.; Tan, X.; Dy, A.J.; Braff, D.; Akana, R.T.; Furuta, Y.; Donghia, N.; Ananthakrishnan, A.; Collins, J.J. A low-cost paper-based synthetic biology platform for analyzing gut microbiota and host biomarkers. *Nat. Commun.* **2018**, *9*, 3347. [CrossRef]
144. Marlicz, W.; Skonieczna-Żydecka, K.; Dabos, K.J.; Łoniewski, I.; Koulaouzidis, A. Emerging concepts in non-invasive monitoring of Crohn's disease. *Ther. Adv. Gastroenterol.* **2018**, *11*, 1756284818769076. [CrossRef] [PubMed]
145. Hua, Z.; Liu, S.; Yang, G.; Hou, X.; Fang, Y. Next-generation probiotics: Innovations in safety assessments. *Curr. Opin. Food Sci.* **2024**, *61*, 101238. [CrossRef]
146. Wei, X.; Xi, P.; Chen, M.; Wen, Y.; Wu, H.; Wang, L.; Zhu, Y.; Ren, Y.; Gu, Z. Capsule robots for the monitoring, diagnosis, and treatment of intestinal diseases. *Mater. Today Bio* **2024**, *29*, 101294. [CrossRef] [PubMed]
147. Chelliah, R.; Wei, S.; Daliri, E.B.-M.; Rubab, M.; Elahi, F.; Yeon, S.-J.; Jo, K.h.; Yan, P.; Liu, S.; Oh, D.H. Development of nanosensors based intelligent packaging systems: Food quality and medicine. *Nanomaterials* **2021**, *11*, 1515. [CrossRef]
148. McCusker, R.H.; Kelley, K.W. Immune–neural connections: How the immune system's response to infectious agents influences behavior. *J. Exp. Biol.* **2013**, *216*, 84–98. [CrossRef]
149. Curry, K.; Nute, M.; Treangen, T.J. It takes guts to learn: Machine learning techniques for disease detection from the gut microbiome. *Emerg. Top. Life Sci.* **2021**, *5*, 815–827. [CrossRef]
150. Sun, Y.; Li, H.; Zheng, L.; Li, J.; Hong, Y.; Liang, P.; Kwok, L.-Y.; Zuo, Y.; Zhang, W.; Zhang, H. iProbiotics: A machine learning platform for rapid identification of probiotic properties from whole-genome primary sequences. *Brief. Bioinform.* **2022**, *23*, bbab477. [CrossRef]
151. Bergamini, C.M.; Bianchi, N.; Giaccone, V.; Catellani, P.; Alberghini, L.; Stella, A.; Biffani, S.; Yaddehige, S.K.; Bobbo, T.; Taccioli, C. Machine learning algorithms highlight trna information content and chargaff's second parity rule score as important features in discriminating probiotics from non-probiotics. *Biology* **2022**, *11*, 1024. [CrossRef]
152. Maftei, N.M.; Raileanu, C.R.; Balta, A.A.; Ambrose, L.; Boev, M.; Marin, D.B.; Lisa, E.L. The potential impact of probiotics on human health: An update on their health-promoting properties. *Microorganisms* **2024**, *12*, 234. [CrossRef]
153. Mazziotta, C.; Tognon, M.; Martini, F.; Torreggiani, E.; Rotondo, J.C. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells* **2023**, *12*, 184. [CrossRef]
154. Berson, E.; Chung, P.; Espinosa, C.; Montine, T.J.; Aghaeepour, N. Unlocking human immune system complexity through AI. *Nat. Methods* **2024**, *21*, 1400–1402. [CrossRef] [PubMed]
155. Kumar, P.; Rajeshwari, S.; Shukla, P. Artificial intelligence and synthetic biology approaches for human gut microbiome. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 2103–2121. [CrossRef] [PubMed]
156. Ozcelik, F.; Dundar, M.S.; Yildirim, A.B.; Henehan, G.; Vicente, O.; Sánchez-Alcázar, J.A.; Gokce, N.; Yildirim, D.T.; Bingol, N.N.; Karanfilska, D.P.; et al. The impact and future of artificial intelligence in medical genetics and molecular medicine: An ongoing revolution. *Funct. Integr. Genom.* **2024**, *24*, 138. [CrossRef]
157. Wu, S.; Feng, T.; Tang, W.; Qi, C.; Gao, J.; He, X.; Wang, J.; Zhou, H.; Fang, Z. metaProbiotics: A tool for mining probiotic from metagenomic binning data based on a language model. *Brief. Bioinform.* **2024**, *25*, bbae085. [CrossRef]
158. Orkkatteri Krishnan, A.; Mudgal, L.N.; Soni, V.; Prakash, T. ProbML: A machine learning-based genome classifier for identifying probiotic organisms. *Mol. Nutr. Food Res.* **2025**, e70025. [CrossRef]
159. Kang, M.; Tian, J. Machine Learning: Data Pre-processing. In *Prognostics and Health Management of Electronics*; John Wiley and Sons: Hoboken, NJ, USA, 2018; pp. 111–130.
160. Varoquaux, G.; Colliot, O. Evaluating machine learning models and their diagnostic value. In *Machine Learning for Brain Disorders*; Humana: New York, NY, USA, 2023; pp. 601–630.
161. Li, P.; Luo, H.; Ji, B.; Nielsen, J. Machine learning for data integration in human gut microbiome. *Microb. Cell Factories* **2022**, *21*, 241. [CrossRef]
162. Sulaimany, S.; Farahmandi, K.; Mafakheri, A. Computational prediction of new therapeutic effects of probiotics. *Sci. Rep.* **2024**, *14*, 11932. [CrossRef]

163. Erion, G.; Janizek, J.D.; Hudelson, C.; Utarnachitt, R.B.; McCoy, A.M.; Sayre, M.R.; White, N.J.; Lee, S.-I. A cost-aware framework for the development of AI models for healthcare applications. *Nat. Biomed. Eng.* **2022**, *6*, 1384–1398. [CrossRef]
164. Choudhary, R.; Mahadevan, R. Toward a systematic design of smart probiotics. *Curr. Opin. Biotechnol.* **2020**, *64*, 199–209. [CrossRef]
165. Pasolli, E.; Truong, D.T.; Malik, F.; Waldron, L.; Segata, N. Machine learning meta-analysis of large metagenomic datasets: Tools and biological insights. *PLoS Comput. Biol.* **2016**, *12*, e1004977. [CrossRef]
166. Wu, J.; Zhang, B.; Liu, X.; Gu, W.; Xu, F.; Wang, J.; Liu, Q.; Wang, R.; Hu, Y.; Liu, J. An intelligent intestine-on-a-chip for rapid screening of probiotics with relief-enteritis function. *Adv. Mater.* **2024**, *36*, 2408485. [CrossRef] [PubMed]
167. Kang, S.-B.; Kim, H.; Kim, S.; Kim, J.; Park, S.-K.; Lee, C.-W.; Kim, K.O.; Seo, G.-S.; Kim, M.S.; Cha, J.M.; et al. Potential oral microbial markers for differential diagnosis of Crohn's disease and ulcerative colitis using machine learning models. *Microorganisms* **2023**, *11*, 1665. [CrossRef] [PubMed]
168. Iqbal, M.J.; Javed, Z.; Sadia, H.; Qureshi, I.A.; Irshad, A.; Ahmed, R.; Malik, K.; Raza, S.; Abbas, A.; Pezzani, R. Clinical applications of artificial intelligence and machine learning in cancer diagnosis: Looking into the future. *Cancer Cell Int.* **2021**, *21*, 270. [CrossRef]
169. Dlamini, Z.; Francies, F.Z.; Hull, R.; Marima, R. Artificial intelligence (AI) and big data in cancer and precision oncology. *Comput. Struct. Biotechnol. J.* **2020**, *18*, 2300–2311. [CrossRef]
170. Freitas, P.; Silva, F.; Sousa, J.V.; Ferreira, R.M.; Figueiredo, C.; Pereira, T.; Oliveira, H.P. Machine learning-based approaches for cancer prediction using microbiome data. *Sci. Rep.* **2023**, *13*, 11821. [CrossRef]
171. Huang, A.; Huo, Y.; Zhong, Y.; Yang, W. AI Technology for Anti-Aging: An Overview. In Proceedings of the 2023 International Conference on Intelligent Supercomputing and BioPharma (ISBP), Zhuhai, China, 6–8 January 2023; pp. 54–62.
172. Wu, Z.; Feng, C.; Hu, Y.; Zhou, Y.; Li, S.; Zhang, S.; Hu, Y.; Chen, Y.; Chao, H.; Ni, Q. HALD, a human aging and longevity knowledge graph for precision gerontology and geroscience analyses. *Sci. Data* **2023**, *10*, 851. [CrossRef]
173. Oniszczuk, A.; Oniszczuk, T.; Gancarz, M.; Szymańska, J. Role of gut microbiota, probiotics and prebiotics in the cardiovascular diseases. *Molecules* **2021**, *26*, 1172. [CrossRef]
174. Tao, Y.-W.; Gu, Y.-L.; Mao, X.-Q.; Zhang, L.; Pei, Y.-F. Effects of probiotics on type II diabetes mellitus: A meta-analysis. *J. Transl. Med.* **2020**, *18*, 30. [CrossRef]
175. Oudat, Q.; Okour, A. The role of probiotics in modulating gut microbiota and metabolic health for weight management: A mini review. *Acta Microbiol. Hell.* **2025**, *70*, 5. [CrossRef]
176. Wan, Y.; Zuo, T.; Xu, Z.; Zhang, F.; Zhan, H.; Chan, D.; Leung, T.-F.; Yeoh, Y.K.; Chan, F.K.; Chan, R. Underdevelopment of the gut microbiota and bacteria species as non-invasive markers of prediction in children with autism spectrum disorder. *Gut* **2022**, *71*, 910–918. [CrossRef]
177. Wang, S.; Naumovski, N.; Ajlouni, S.; Ayyash, M.; Silva, R.; Balthazar, C.F.; Esmerino, E.A.; Freitas, M.Q.; da Silva, M.C.; Sant'Ana, A.S. Nonbovine milk and its products as sources of probiotics delivery: An overview of its viability, functionality and product quality characteristics. *Int. J. Dairy Technol.* **2023**, *76*, 482–511. [CrossRef]
178. Ge, S.; Han, J.; Sun, Q.; Zhou, Q.; Ye, Z.; Li, P.; Gu, Q. Research progress on improving the freeze-drying resistance of probiotics: A review. *Trends Food Sci. Technol.* **2024**, *147*, 104425. [CrossRef]
179. Nguyen, T.H.; Kim, J.-S.; Kwon, H.-J.; Kang, C.-H. The effect of a glutathione (gsh)-containing cryo-protectant on the viability of probiotic cells using a freeze-drying process. *Fermentation* **2023**, *9*, 187. [CrossRef]
180. Chen, H.; Tian, M.; Chen, L.; Cui, X.; Meng, J.; Shu, G. Optimization of composite cryoprotectant for freeze-drying *Bifidobacterium bifidum BB01* by response surface methodology. *Artif. Cells Nanomed. Biotechnol.* **2019**, *47*, 1559–1569. [CrossRef] [PubMed]
181. Kavak, A.E.; Zent, İ.; Özdemir, A.; Dertli, E. Optimization of cryoprotectant formulation to enhance the viability of *Lactiplantibacillus plantarum NBC99* isolated from human origin. *Prep. Biochem. Biotechnol.* **2024**, *54*, 958–966. [CrossRef]
182. Yusuff, A.S.; Ishola, N.B.; Gbadamosi, A.O. Artificial intelligence techniques and response surface methodology for the optimization of methyl ester sulfonate synthesis from used cooking oil by sulfonation. *ACS Omega* **2023**, *8*, 19287–19301. [CrossRef]
183. Bozkurt Keser, S.; Buruk Sahin, Y. Response surface methodology to tune artificial neural network hyper-parameters. *Expert Syst.* **2021**, *38*, e12792. [CrossRef]
184. Gul, L.B.; Gul, O.; Yilmaz, M.T.; Dertli, E.; Con, A.H. Optimization of cryoprotectant formulation to enhance the viability of *Lactobacillus brevis ED25*: Determination of storage stability and acidification kinetics in sourdough. *J. Food Process. Preserv.* **2020**, *44*, e14400. [CrossRef]
185. Bobba, S.; Harguindeguy, M.; Colucci, D.; Fissore, D. Diffuse interface model of the freeze-drying process of individually frozen products. *Dry. Technol.* **2020**, *38*, 758–774. [CrossRef]
186. Van Chuyen, H.; Le Minh, T.; Phu, Q.P.; Giurgiulescu, L.; Tan, D. Mathematical model study to optimize the freeze drying process for production of dried yogurt. *Carpathian J. Food Sci. Technol.* **2024**, *16*, 151–163.
187. Zhu, H.; Jin, D.; Fu, N.; Chen, X.D.; Xiao, J. Neural network modeling of the dynamic inactivation of probiotics during single droplet drying for improved cell viability. *Powder Technol.* **2023**, *413*, 118042. [CrossRef]

188. Chutrtong, J.; Kularbphettong, K.; Sirichokworrakit, S. Investigation into the impact of varying inulin levels on the survival of probiotic microorganisms. In Proceedings of the 2023 International Conference on Computational Science and Computational Intelligence (CSCI), Las Vegas, NV, USA, 13–15 December 2023; pp. 577–581.
189. Reker, D. Practical considerations for active machine learning in drug discovery. *Drug Discov. Today Technol.* **2019**, *32*, 73–79. [CrossRef] [PubMed]
190. Chourasia, R.; Abedin, M.M.; Chiring Phukon, L.; Sahoo, D.; Singh, S.P.; Rai, A.K. Biotechnological approaches for the production of designer cheese with improved functionality. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 960–979. [CrossRef]
191. Yang, S.; Bai, M.; Kwok, L.-Y.; Zhong, Z.; Sun, Z. The intricate symbiotic relationship between lactic acid bacterial starters in the milk fermentation ecosystem. *Crit. Rev. Food Sci. Nutr.* **2025**, *65*, 728–745. [CrossRef]
192. Yang, S.; Bai, M.; Liu, W.; Li, W.; Zhong, Z.; Kwok, L.-Y.; Dong, G.; Sun, Z. Predicting *Lactobacillus delbrueckii* subsp. *bulgaricus*-*Streptococcus thermophilus* interactions based on a highly accurate semi-supervised learning method. *Sci. China Life Sci.* **2025**, *68*, 558–574. [CrossRef]
193. Hernández Medina, R.; Kutuzova, S.; Nielsen, K.N.; Johansen, J.; Hansen, L.H.; Nielsen, M.; Rasmussen, S. Machine learning and deep learning applications in microbiome research. *ISME Commun.* **2022**, *2*, 98. [CrossRef]
194. Oh, M.; Zhang, L. DeepMicro: Deep representation learning for disease prediction based on microbiome data. *Sci. Rep.* **2020**, *10*, 6026. [CrossRef]
195. Yang, J.; Shin, T.-S.; Kim, J.S.; Jee, Y.-K.; Kim, Y.-K. A new horizon of precision medicine: Combination of the microbiome and extracellular vesicles. *Exp. Mol. Med.* **2022**, *54*, 466–482. [CrossRef]
196. Hao, X.; Xia, Y.; Wang, Y.; Zhang, X.; Liu, L. The addition of probiotic promotes the release of ACE-I peptide of Cheddar cheese: Peptide profile and molecular docking. *Int. Dairy J.* **2023**, *137*, 105507. [CrossRef]
197. Ul Ain, N.; Naveed, M.; Aziz, T.; Shabbir, M.A.; Al Asmari, F.; Abdi, G.; Sameeh, M.Y.; Alhhazmi, A.A. Mix-match synthesis of nanosynbiotics from probiotics and prebiotics to counter gut dysbiosis via AI integrated formulation profiling. *Sci. Rep.* **2024**, *14*, 18397. [CrossRef]
198. Cunningham, M.; Azcarate-Peril, M.A.; Barnard, A.; Benoit, V.; Grimaldi, R.; Guyonnet, D.; Holscher, H.D.; Hunter, K.; Manurung, S.; Obis, D. Shaping the future of probiotics and prebiotics. *Trends Microbiol.* **2021**, *29*, 667–685. [CrossRef] [PubMed]
199. Liao, W.; Shen, J.; Manickam, S.; Li, S.; Tao, Y.; Li, D.; Liu, D.; Han, Y. Investigation of blueberry juice fermentation by mixed probiotic strains: Regression modeling, machine learning optimization and comparison with fermentation by single strain in the phenolic and volatile profiles. *Food Chem.* **2023**, *405*, 134982. [CrossRef] [PubMed]
200. Martinović, A.; Martinović, I.; Mora, D.; Arioli, S. Uncovering the Probiotic supplement landscape: Market offerings, sales patterns, and future forecasts using machine learning approach—A case study of montenegro. *Probiotics Antimicrob. Proteins* **2024**, 1–24.

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