

ADVANCEMENTS IN PROBIOTIC ISOLATION AND CHARACTERIZATION: METHODS, MICROBIAL DIVERSITY, AND HEALTH BENEFIT

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ABSTRACT

Probiotics are live microbes that when administered in small amounts confer some health benefits to the host. Several different types of products which are formulated as drugs or other dietary supplements, originate from live microbes called probiotics. The majorities of these organisms belong to the LAB (Lactic Acid Bacteria) group. The LAB predominantly includes organisms which belong to the genera of *Lactobacillus* and *Bifidobacterium* but from varied species and strains which altogether is considered as potential probiotic. The aim of this review is to comprehensively explore the methodologies and advancements in the isolation and characterization of probiotics. This review investigates different methods for separating probiotics from a range of sources, including fermented foods, the human microbiome, and natural settings. It highlights the importance of cutting-edge methods like molecular biology, metagenomics, and whole genome sequencing in the identification of new probiotic strains, the

description of their genetic and functional characteristics, and the evaluation of their possible health advantages.

Keywords: *probiotics, isolation, characterization, microbiome, lactobacillus*

1. Introduction

Probiotics have attracted a lot of attention in the last several years because of their possible health advantages and range of uses in the food, pharmaceutical, and agricultural industries. Probiotics are live bacteria that, when given in sufficient quantities, boost the host's health. Probiotics have the potential to be used therapeutically because of their capacity to alter gut microbiota, boosting immunity, and having antimicrobial properties. The notion of probiotics originated in the early 1900s, when Elie Metchnikoff said that lactic acid bacteria-containing fermented dairy products could extend life and improve health [20]. Subsequent research has clarified the various pathways via which probiotics achieve their benefits on human body. Probiotics were once primarily linked to digestive health, but their uses have since broadened to include dermatological, oral health, and even mental wellness [35].

Probiotics come from many different sources, each of which has its own special strains with certain health advantages. Probiotics are mostly found naturally in fermented foods and drinks such kombucha, yogurt, kefir, sauerkraut, and kimchi. Lactic acid bacteria (LAB) and other helpful microorganisms process these traditional meals, populating the gut microbiota after ingestion and supporting digestive health and general well-being [24]. To find probiotic strains with desired health-promoting qualities, it is essential to isolate and characterize the organisms. By employing selective culture conditions and biochemical assays, probiotic bacteria were traditionally identified from fermented foods and human faecal samples. Probiotic identification and characterisation, however, has been completely transformed by advances in genomics and molecular biology. The genetic makeup, metabolic pathways, and possible modes of action of probiotic strains can be fully characterized by the use of techniques including

transcriptomics, metagenomics, and whole-genome sequencing.

Probiotics' ability to support digestive health is one of its main advantages. They assist in preserving a balanced population of gut bacteria, which helps lessen the symptoms of digestive problems such as diarrhoea brought on by antibiotic use, inflammatory bowel diseases (IBD), and irritable bowel syndrome (IBS)[13]. Additionally, probiotics' immunomodulatory effects have been researched. These effects may improve immune responses and lessen the frequency and severity of allergies and respiratory infections. Furthermore, they could enhance lipid profiles and glucose metabolism, which could help manage diseases like type 2 diabetes and obesity by promoting metabolic health [31]. This review paper examines how probiotics are isolated and characterized, covering traditional and modern methods. The study highlights the potential of probiotics to promote health and prevent disease by discussing their significance in food and medicine, among other applications. It also discusses future research paths that will improve the safety, efficacy, and customized uses of probiotics in healthcare.

1. Sources of probiotics

It has long been known that fermented foods are a great source of probiotics. One of the most popular foods high in probiotics is yogurt, which include strains of *Lactobacillus acidophilus* and *Bifidobacterium lactis*, which support immune system and digestion and hence promote gut health [19]. Along with helping with lactose digestion, kefir, a fermented milk beverage, has a rich probiotic profile due to the wide community of bacteria and yeasts it contains. Traditional fermented foods like kimchi, kombucha, and sauerkraut include a range of probiotic strains, including *Saccharomyces boulardii* and *Lactobacillus plantarum*, which are well-known for their immunomodulatory and antibacterial qualities [30].

The microbiota found in the faeces of humans is a storehouse of various microbial communities and a useful resource for the discovery of probiotics. The therapeutic potential of transplanting beneficial bacteria from healthy donors to

recipients suffering from gastrointestinal illnesses has been highlighted by research on faecal microbiota transplantation (FMT) [40]. Beyond FMT, research is still being done to find and describe new probiotic strains from human sources that have health-promoting qualities, like immune modulation and maintenance of gut barrier integrity. Probiotics obtained from natural sources, like plants, soil, and aquatic environments, increase the variety of microbial strains that can be used for both nutritional and medicinal purposes. For example, the ability of soil bacteria to withstand adversity and offer health advantages similar to those of typical probiotics found in fermented foods has been studied [27]. Probiotics that have been isolated from marine settings also have special adaptations and bioactive substances that may have positive effects on health, such as anti-inflammatory and antioxidant qualities.

Table 1: Some common probiotic strains and their sources

<i>Species</i>	Source	Reference
<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i>	Kefir	[22]
<i>Lactobacillus rhamnosus</i>	Kombucha	[27]
<i>Lactobacillus paracasei</i> , <i>Lactobacillus casei</i>	F e r m e n t e d soybeans	[36]
<i>Lactobacillus plantarum</i> , <i>Lactobacillus brevis</i>	Kimchi	[42]
<i>Lactobacillus lactis</i> , <i>Lactobacillus plantarum</i>	Idli batter	[33]
<i>Lactobacillus acidophilus</i>	Miso	[14]
<i>Lactobacillus helveticus</i>	Parmigiano cheese	[4]
<i>Lactobacillus plantarum</i> , <i>Lactobacillus bulgaricus</i>	Koumiss	[29]

<i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i>	Kalari cheese	[21]
<i>Lactobacillus brevis</i>	Sauerkraut	[19]
<i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i>	Yoghurt	[12]
<i>Lactobacillus lactis</i>	Buttermilk	[2]
<i>Lactobacillus casei</i> , <i>Bifidobacterium breve</i>	Tempeh	[5]

2. Techniques for isolation and characterization of probiotics

The isolation and characterization of probiotics is a key step in microbiology and biotechnology. Microbial strains from a variety of ecological niches, such as fermented foods, the human microbiota, and natural settings, are systematically explored and chosen for this purpose. The use of selective media and conditions to isolate specific probiotic species, is essential in traditional culture-based approaches [10]. Simultaneously, the utilization of novel species and thorough microbial profiling has been made possible by the development of molecular tools like 16S rRNA gene sequencing and metagenomics [38]. These approaches are essential for clarifying the mechanisms of action of probiotics as well as their capacity to alter the makeup of the gut microbiota, strengthen the immune system, and generate bioactive metabolites that are vital to human health.

2.1 Traditional culture-based methods for isolation

Conventional culture-based techniques are used to carefully isolate probiotics from a variety of natural sources, namely fermented foods, and human microbiota. To guarantee the identification and selection of advantageous microbial strains, traditional culture-based techniques involve several crucial procedures. Usually, the first step in these procedures is the extraction of samples from sources. Following that, the samples are selectively enriched in specialized growth medium that promote the development of the intended

probiotic species while inhibiting the growth of rival bacteria. Formulations for selective media, such as Bifidobacterium-selective media or MRS agar for lactobacilli, are created with certain nutrients and pH levels that promote the growth of probiotic strains [6]. Following identification of isolated colonies based on their physical traits, biochemical assays, such as carbohydrate fermentation profiles and enzyme activity, are used to confirm the isolates. Moreover, for accurate species identification and phylogenetic analysis, contemporary methods like PCR and sequencing of conserved genes (such as 16S rRNA) are used [39]. Because of their ease of use, dependability, and affordability in identifying potential candidates for additional research and use in functional foods and therapeutic interventions, traditional culture-based methods are still valuable for the initial stages of probiotic discovery and strain characterization, even in light of the advancements in molecular and genomic approaches.

2.2 Molecular techniques for isolation and characterization

Molecular methods for isolating probiotics have transformed biotechnology and microbial ecology by making it possible to precisely identify, characterize, and utilize advantageous microbes. In contrast to conventional culture-based techniques, molecular approaches provide a thorough understanding of the diversity and functionality of microbes. The direct analysis of genetic material from environmental samples has been made possible by methods like metagenomics. It has produced novel probiotic candidates from a variety of environments, including the human gut and natural habitats [16]. Moreover, 16S rRNA gene sequencing has been a fundamental instrument for taxonomic categorization and microbial community characterization, making it easier to identify certain probiotic strains and their quantity. Along with these, methods like whole genome sequencing (WGS) reveal the complete genetic composition of probiotic bacteria and throw light on their detailed metabolic pathways.

3.2.1 16s rRNA Sequencing

A crucial molecular technique for the isolation and identification of probiotics, 16S rRNA sequencing offers information on the

diversity of microorganisms and taxonomic classification according to sequence differences in the 16S rRNA gene, a region that is universally conserved in bacteria. Researchers can analyse complex microbial communities, including those containing potentially probiotic species, using this approach [25]. 16S rRNA sequencing makes it easier to precisely identify particular bacterial strains and their abundance in intricate microbial ecosystems for probiotic research. Using taxonomic classification and functional predictions obtained from genomic data, this technique has proven useful in the discovery of novel probiotic candidates and the assessment of their potential health effects [7]. Moreover, 16S rRNA sequencing offers a basis for understanding the ecological functions of probiotics in diverse settings, microbial community dynamics, and probiotic-host interactions.

3.2.2 Metagenomics

Metagenomics is a powerful approach in microbiology that involves the direct study of genetic material recovered from environmental samples, bypassing the need for microbial cultivation. This technique allows researchers to explore the collective genomes of entire microbial communities, including previously unculturable species, thereby providing a comprehensive view of microbial diversity and functionality [39]. In the context of probiotics, metagenomic studies have been instrumental in identifying novel microbial candidates from diverse environments such as soil, marine habitats, and the human gut microbiota. These studies not only expand the scope of study of potential probiotic species but also offer insights into their ecological roles and functional properties.

3.2.3 Whole genome sequencing (WGS)

Whole genome sequencing (WGS) has emerged as a key component in the isolation and characterisation of these organisms because of its ability to provide a thorough understanding of the genetic makeup, activity, and possible health advantages of probiotics. WGS offers a thorough analysis of the complete genome of microbial strains, including genes encoding for metabolic pathways, virulence factors, and probiotic features [1]. This is in contrast to conventional

culture-based methods and partial sequencing techniques like 16S rRNA sequencing. The first step in the WGS method is to harvest high-quality genomic DNA from the target probiotic strain. Then, using cutting-edge technologies like Illumina or PacBio, which produce enormous volumes of sequence data, this DNA is broken up and libraries are ready for sequencing. The entire genetic code of the probiotic strain can then be examined by researchers by using bioinformatics methods to put these sequences together into complete genomes[16].

3.2.4 Functional genomics and transcriptomics

Functional genomics and transcriptomics are pivotal in the isolation and characterization of probiotics, offering detailed insights into their genetic and functional attributes. These methodologies enable the comprehensive study of gene expression profiles, metabolic pathways, and regulatory networks within probiotic microorganisms. The methodical investigation of gene activities and their connections with the genome is known as functional genomics. Genes encoding probiotic characteristics like resilience to environmental stressors, adhesion to host tissues, and antimicrobial synthesis can be identified using methods like comparative genomics and whole genome sequencing (WGS) [28]. Researchers can forecast the possible health-promoting qualities of probiotic strains and maximize their utility in therapeutic applications by studying their complete genetic composition.

Transcriptomics is concerned with the analysis of the transcriptome, which is the whole collection of RNA transcripts generated by the genome at particular growth stages or environmental circumstances. Researchers can measure the levels of gene expression, find genes that are differentially expressed, and discover the regulatory networks that mediate probiotic actions by using RNA sequencing [23]. This method makes it easier to create probiotic therapies that are suited to certain medical diseases by offering dynamic insights into how bacteria react to different environmental stimuli and interact with host cells.

2.3 Techniques for biological and phenotypic characterization

Probiotics are biologically characterized by assessing physiological characteristics such as resistance to gastrointestinal disorders, adhesion to intestinal epithelial cells, and tolerance to acid and bile. The ability to survive and colonize in the gut environment is essential for probiotic efficacy. In vitro assays, flow cytometry, and microscopy are among the methods used to evaluate probiotic survival and adherence in simulated gastrointestinal environments [15]. The study of probiotic metabolic processes, such as the synthesis of vitamins, short-chain fatty acids, and antibiotic chemicals, is included in phenotypic characterisation. Their possible health-promoting qualities and interactions with the host microbiota are facilitated by these metabolic outputs [3].

In biological characterisation, the following physiological characteristics are crucial in determining the survivability and usefulness of probiotics in the gastrointestinal tract:

- i. **Acid and Bile Tolerance:** For probiotics to be effective, they must be able to withstand exposure to bile salts in the intestines and acidic environments in the stomach. Probiotics can be assessed by subjecting them to artificial stomach and intestinal fluids in order to gauge their viability and tolerance [13].
- ii. **Adherence to Intestinal Epithelial Cells:** In order for probiotics to colonize and remain in the gut mucosa, they must adhere to intestinal epithelial cells. This allows them to engage with the host immune system and affect the makeup of the microbiota [15]. Adhesion assays are used to evaluate the adhesion properties of probiotics utilizing intestinal cell lines or cell culture models.

Phenotypic characterization explores the metabolic activities and functional behaviours of probiotics that contribute to their health-promoting effects:

- i. **Formation of antimicrobial compounds:** A variety of probiotics generate compounds that inhibit pathogenic bacteria and improve gut health, including bacteriocins,

organic acids, and hydrogen peroxide [8]. Antimicrobial activity tests against certain pathogens are used to evaluate these substances.

ii. Study of metabolic routes: Knowledge of the metabolic routes of probiotics, such as the fermentation of dietary fibres to short-chain fatty acids (SCFAs), sheds light on how these microbes contribute to better nutrition metabolism and gut barrier function [18]. The profiles and activity of metabolic pathways are revealed by metabolic profiling methods like mass spectrometry and NMR spectroscopy.

2.4 Techniques for functional and clinical characterization

Through clinical trials and in vivo models, functional characterisation evaluates the unique health advantages of probiotics. Studies like this assess the effectiveness of probiotics in treating ailments like immunological regulation, allergies, and gastrointestinal diseases. The processes behind probiotic effects on human health are explained by measuring biomarkers of host response, makeup of the gut microbiota, and metabolic profiles [41]. Clinical trials are conducted on humans to evaluate the effectiveness of probiotics in treating gastrointestinal problems such as diarrhoea and inflammatory bowel disease, avoiding infections by pathogen competitive exclusion, and reducing allergy symptoms via immunological modulation [9]. Probiotic goods must adhere to regulatory standards, and safety assessments guarantee this by highlighting the GRAS (Generally Recognized as Safe) status of the products [18]. These studies add to the body of evidence supporting the use of probiotics in personalized medicine and functional foods by examining their safety profiles as well as their effects on general immunological and metabolic health.

3. Future scope and challenges

Innovative research approaches and developing technology are expected to produce considerable breakthroughs in the isolation and characterization of probiotics in the future. It is anticipated that isolation techniques would advance beyond conventional culture-based procedures to integrate high-throughput screening techniques, like robotic automation

and microfluidics, allowing for the quick identification and production of a variety of probiotic strains from intricate microbial ecosystems [34]. Probiotic discovery is expected to be revolutionized by metagenomic and single-cell genomics techniques, which will enable researchers to investigate unculturable microbial ecosystems and identify particular beneficial bacteria with focused health effects.

Molecular, cellular, and community-level understanding of probiotic functioning requires characterization techniques to depend more and more on multi-omics approaches that integrate data from genomes, transcriptomics, proteomics, and metabolomics [27]. As metabolomics advances, it will become clearer how probiotics metabolically function, particularly how they produce bioactive substances like vitamins and short-chain fatty acids that are essential for immune regulation and gut health. Understanding the gene expression patterns and protein profiles of probiotics under different physiological situations through transcriptomics and proteomics will shed light on their methods of action in relation to adhesion, colonization, and host cell interaction [32].

Probiotics are also anticipated to find more use in the treatment of neurological conditions, metabolic diseases, and customized nutrition, in addition to gastrointestinal health issues. Thorough clinical trials utilizing cutting-edge molecular techniques will help to confirm the effectiveness of probiotics, adjust dosing schedules, and create safety profiles specific to certain demographics and medical conditions [26].

Despite technical developments, there are still significant hurdles in the isolation and characterisation of probiotics. Robust techniques are needed to recognize and isolate probiotic strains appropriate for a range of demographics due to the cultural variety in gut microbiota [13]. To maintain consistency in quality and health benefits, probiotic formulation standardization and efficacy testing are still essential. Strict adherence to worldwide criteria and comprehensive clinical trials are required to meet regulatory requirements for safety and efficacy [11]. To overcome these obstacles and create guidelines for the creation and marketing

of probiotic products that adhere to strict safety and efficacy criteria, cooperation between academic institutions, business, and regulatory agencies is crucial.

4. Conclusion

Although there has been progress in the field of isolating and characterizing probiotics, more research and technical developments are expected to drive further development and application in a range of medical and health concerns. Because of advancements in molecular techniques such as metagenomics and single-cell genomics, probiotic strains may now be isolated from a wide range of habitats, a departure from the old culture-based procedures. Our knowledge of probiotic activity at the molecular level has been expanded by characterization techniques that span from morphological and physiological evaluations to omics technologies including proteomics, metabolomics, transcriptomics, and genomes. The functions of probiotics in immunological control, metabolic regulation, gut health, and other areas have been clarified by these findings. Their potential therapeutic applications in the management of gastrointestinal illnesses, immune function enhancement, and possible influence on systemic health conditions demonstrate the growing clinical relevance of these microorganisms. But issues like population-specific variations in probiotic efficacy, legal requirements for supporting health claims, and moral questions about probiotic promotion and consumer education still need to be addressed.

Future avenues in research should focus on customized probiotic treatments based on each person's unique microbiome composition, utilizing developments in precision medicine and analytics. Understanding how probiotics interact with the gut microbiota and host physiology can help to maximize the safety and effectiveness of probiotics. Moreover, overcoming these obstacles and successfully integrating research findings into clinical practice would need cooperative efforts across scientific fields, regulatory agencies, and industry sectors. In conclusion, continued research and interdisciplinary collaborations promise to advance the field and pave the way for individualized, evidence-based probiotic interventions that improve health

outcomes globally, even though there is still much to learn about the full therapeutic potential of probiotics and their wider impact on human health.

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