Asian Journal of Pharmaceutical and Health Sciences
http://www.ajphs.com
ISSN: 2231- 2331(Print) | ISSN: 2231–234X(Online)

Review article

Probiotic Strain Identification: Current and Future Approaches

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Article History
Received :20.12.2025
Revised :14.01.2026
Accepted :26.01.2026

DOI

10.5530/ajphs.2026.16.88

Keywords
Probiotic strain identification
Strain identification approaches
Probiotics

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ABSTRACT

Health benefits of Probiotic are highly strain-specific, making accurate strain identification essential for safety, efficacy, correct labeling, and regulatory compliance. Over time, identification approaches have evolved from traditional culture-based and biochemical methods to advanced molecular and genomic techniques. Current methods include phenotypic and biochemical analyses, strain typing (PFGE, RAPD, MLST), mass-based fingerprinting (MALDI-TOF MS), targeted molecular assays (strain-specific PCR, qPCR, digital PCR), and high-resolution genomic tools such as 16S rRNA gene sequencing, whole-genome sequencing (WGS), and metagenomics. These techniques vary in resolution, turnaround time, cost, and suitability for clinical trials or industrial applications. Despite significant progress, challenges remain in distinguishing closely related strains, detecting low-abundance strains in complex samples, database standardization, nomenclature, and regulatory harmonization. Emerging approaches such as pangenome analysis, long-read sequencing, functional marker discovery, mobile genomic platforms, and AI-driven genomic analysis are expected to overcome current limitations and shape the future of probiotic strain identification. This review mentions the kind of extensive analysis of the present and emerging techniques of identifying the bacterial strain of probiotics, along with the technological constraints and innovative developments in the coming years in the sector.

1. INTRODUCTION

Strain-specific identity is the basis for proving the safety, efficacy, and legality of probiotics. Modern probiotic products comprise strains with high similarities, which vary in their genetic and functional properties. Therefore, classic phenotyping is not an effective technique to ensure strain-specific identity and avoidance of incorrect labeling. In current probiotic science, the focus has moved from species-specific functionality to the high-strain specificity of health properties. Even within the same species, Lactobacillus, Bifidobacterium, or Bacillus strains can exhibit distinctive genome-specific properties, metabolic functions, and biological activities (Binda et al., 2020; Liu et al., 2024). Misidentification is still an internationally observed problem, with variations between labeled and real strain composition of commercial products (Liu et al., 2022; Sarita et al., 2025). It might lead to lower therapeutic advantages,

loss of consumer confidence, and possibly pose risks to public health in terms of virulence and antimicrobial resistance genes in unknown strains. Thus, strain-level authentication has been an operational imperative in markets supervised by regulatory agencies such as the FDA, EFSA, and ISO. Probiotics have gained acceptance in terms of forms of dose regimens. One basic concept in probiotics is that health advantages remain specific on a strain-by-strain basis. Beneficial effects demonstrated by a specific strain cannot be extrapolated to other strains belonging to the same genus or species (Sanders et al., 2018; Hill et al., 2014). Thus, precise identification is essential in establishing efficiency, safety, and successful replication in test results. Along the same line, any form of mislabeling has been rigorously reported in commercial probiotics. There is an evident imperative in terms of serious identification strategies (Morovic et al., 2016; Patro et al., 2016).

Traditionally, the identification of probiotics relied on classical morphological and biochemical tests, such as fermentation patterns of carbohydrates and API-based phenotypic variability. Although these techniques are useful for preliminary differentiation, they lacked sufficient discriminative power to distinguish closely related strains, besides variability due to growth conditions and phenotypic plasticity also being an issue (Salvetti et al., 2012). Molecular tools, in turn, such as 16S rRNA gene sequencing, came to improve species-level resolution but still could not be applied for distinguishing strains because of a very high degree of sequence conservation among many probiotic taxa, such as *Lactobacillus* and *Bifidobacterium* species (Felis & Dellaglio, 2007). Genomic and proteomic technologies have become crucial to the rapid development of molecular microbiology, ushering in a new era for probiotic taxonomy and enabling more detailed characterization, as discussed in the review by Salvetti and O'Toole (2017). The concentration of efforts in polyphasic approaches for identification has combined genotypic, phenotypic, and functional characterizations that ensure reproducibility, authenticity, and translatable results. The discipline has thus been revolutionized by the developments in the fields of genomics, proteomics, and high-resolution molecular typing. Contemporary approaches based on whole-genome sequencing (WGS), strain-specific PCR, multi-locus sequence typing (MLST), and MALDI-TOF mass spectrometry have been found to provide greater accuracy, rapidity, and reproducibility (Majhenič et al., 2017). Among these new approaches, WGS has been recognized as the reference technique of choice to achieve definitive strain identification. It provides high-resolution access to core and accessory genomes, genomic mobile elements, and putative virulence factors or antimicrobial resistance genes (Liu et al., 2025). On the other hand, metagenomics and meta-transcriptomics have been opening new avenues to quantify probiotic strains within mixed cultures of microbes found in food and human gut microbiota without requiring previous culture isolation (Almeida et al., 2019; Quince et al., 2017). However, some challenges remain, and these involve incomplete reference databases, the issue of consistency concerning nomenclature, as well as the need to have a standardized workflow for the regular quality control analysis and reporting. There seems to be a huge potential for improvement of identification and functionality knowledge by joining approaches of long-read sequencing, AI-based bioinformatics analysis, and the development of molecular markers defined by the concept of the pangenome. This review aims to bring together existing approaches, shortfalls, and new technologies and provide a glimpse of the future with respect to developing harmonized standards for identifications of probiotics.

2. METHODOLOGY

This research study was carried out in the form of a systematic and extensive literature review. Scientific literature articles, reviews, and publications relevant to the research subject were searched on various e-databases like Google Scholar, PubMed Central, Scopus, Web of Science, and AI-assisted literature platforms. Searches were performed using combinations of keywords related to probiotics, historical evolution of probiotics, strain identification, molecular techniques, whole-genome sequencing, and strain-specific functionality. Following the initial search, article titles and abstracts were screened to assess relevance. Subsequently, full-text articles were evaluated in detail to determine their suitability for inclusion, and manual screening of reference lists from relevant and highly cited publications was performed to identify additional seminal studies.

3. HISTORICAL EVOLUTION OF PROBIOTIC STRAIN IDENTIFICATION

3.1. Early Phenotypic Taxonomy

Figure 1 shows the chronological progression in the identification of probiotic strains. Until molecular approaches were widely developed, microbial taxonomy, including the classification of probiotics, was largely based on phenotypic properties.

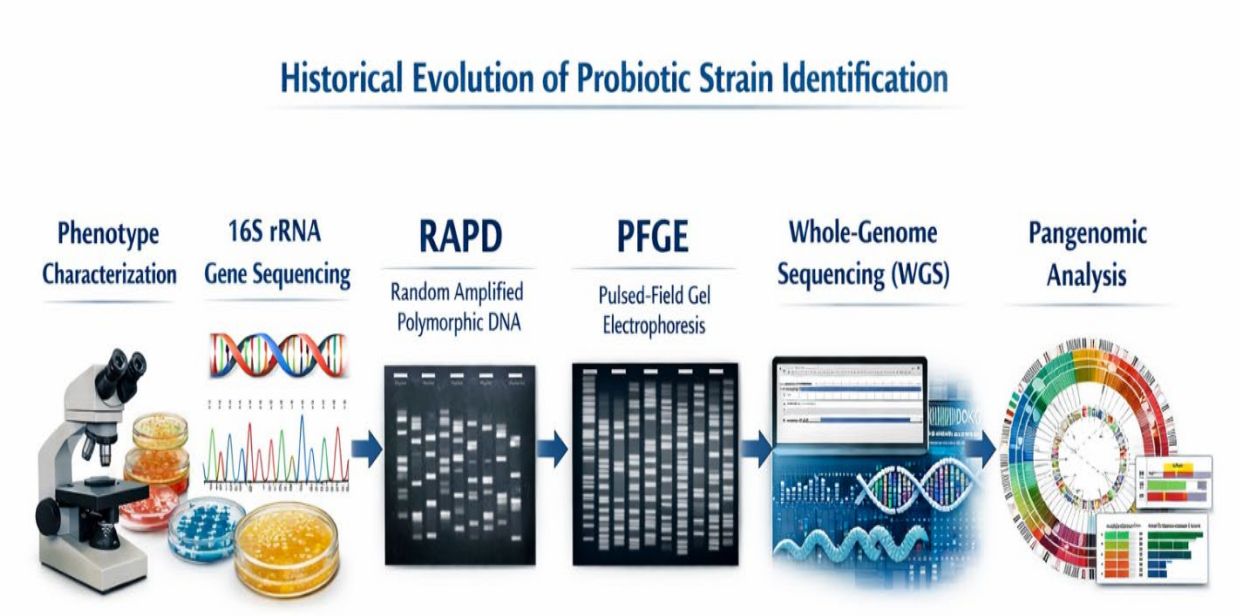

Figure 1: Historical Evolution of Probiotic Strain Identification

It was morphological in nature, combined with other cultural characteristics such as staining property, growth patterns, and biochemical reactions (Vos & Didelot, 2009). The early taxonomists used simple morphological characteristics such as cell shape (rod cells/coccus cells), cell arrangement (chain formation, clusters), Gram reaction, presence of spores, and motility patterns (Mattarelli et al., 2012). These characteristics helped in the initial discrimination of lactic acid bacteria (LAB) and Bifidobacterium species from other genera found in the human intestine. Even these characteristics lacked discriminatory power to distinguish between species and strains (Kandler & Weiss, 1986). Physicochemical characteristics like temperature range, resistance to oxygen, pH range, and tolerance to salts proved to be vital initial markers. LAB bacteria, for instance, could be differentiated based on their resistance to acetic acid and their fermentative growth under anaerobic conditions (Hammes & Vogel, 1995). Biochemical tests, on the other hand, proved to be cornerstones of phenotypic characterization. Tests included carbohydrate fermentation, enzymatic activity (catalase, protease, and lipase tests), gas production from glucose, and production of optically active lactic acid. These tests proved particularly useful for distinguishing the *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* spp. (Scardovi, 1986; Carr et al., 2002). These characteristics remained significantly affected by culture medium, test conditions, and flexibility exhibited in catabolic pathways and these tests proved less reproducible in different laboratories and remained less ideal for strain-level characterization (Felis & Dellaglio, 2007). Around the middle of the last century, techniques involving serotyping, peptidoglycans present within cell walls, analysis of methyl ester of fatty acids (FAME), and menaquinones gained importance. These chemotaxonomic approaches further improved species differentiation but were less sensitive than molecular techniques (Vandamme et al., 1996).

3.2. Emergence of Molecular Microbiology

A major turning point in the characterization of probiotic microorganisms shifted from the classical phenotypic method of taxonomy to the application of molecular microbiology. As of the late 20th century, the use of phenotypic characteristics had become increasingly seen as inadequate for the differentiation of closely related bacteria, particularly in the case of lactic acid bacteria. This is owing to the instability of these characteristics and sensitivity to the environment (Stackebrandt and Goebel, 1994; Vandamme et al., 1996). The discovery and application of the 16S rRNA gene sequencing revolutionized the analysis of bacteria. This method offers the advantage of a universal and phylogenetic characterization of the data (Janda and Abbott, 2007). Conserved areas permitted broad primers, and the highly variable domains permitted the differentiation even at the species level (Woese, 1987). This method established the identification of the genera Lactobacillus, Bifidobacterium, Bacillus, and Streptococcus, which formed the molecular framework for the early taxonomy of probiotics (Salvetti et al., 2012; Zheng et al., 2020). However, the 16S rRNA gene sequencing remained inadequate for the differentiation of strains and closely related species,

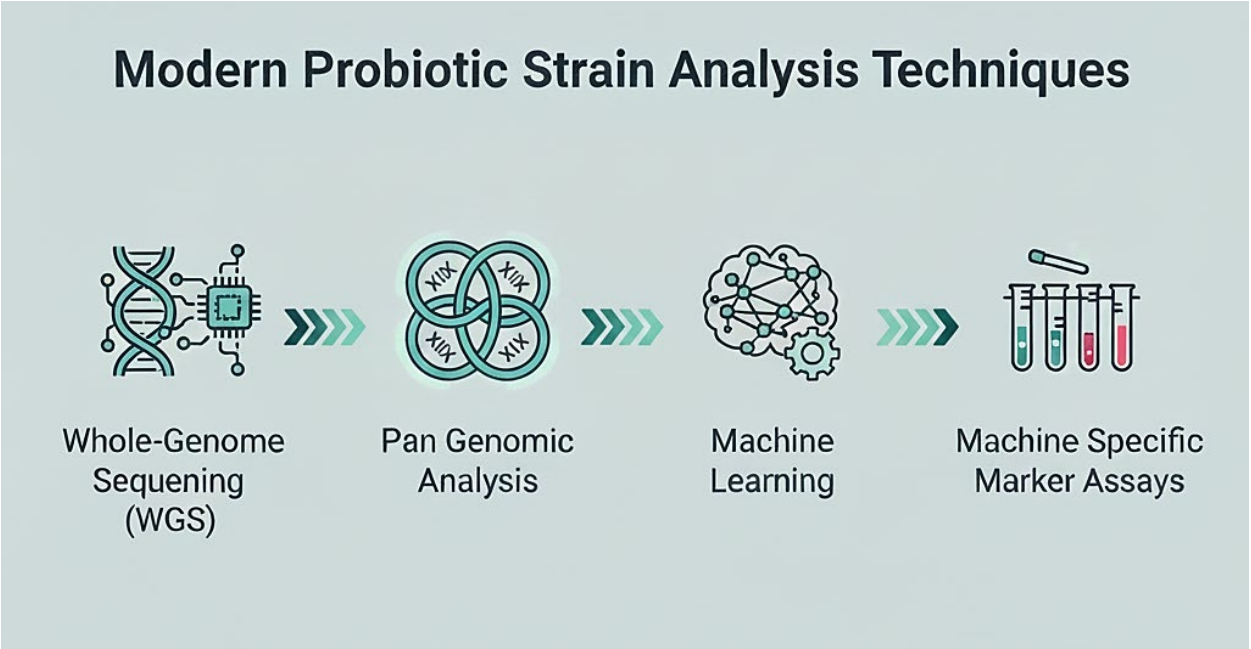

Figure 2: Modern Probiotic Strain Analysis Techniques

and thus, there was a need for an alternative method of molecular analysis. For decades, the DNA-DNA hybridization (DDH) remained the gold standard of the degree of species characterization, and thus, the ≥70% of similarity represented the borderline of the species (Wayne et al., 1987). DDH resolved the taxonomy of several genera, including the *Lactobacillus casei* group and several *Bifidobacterium* spp. Thus, facilitating the integration of the genomic and phenotypic data helps to unify phenotypic and genomic classifications (Felis and Dellaglio, 2007). However, the DDH remained technically complex and not easy to reproduce. The discovery of PCR technology paved the way for the detection of probiotics at the species and strain level. RFLP, ARDRA, and Ribotyping were the earliest genotypic fingerprinting (Giraffa et al., 2000). Random amplified polymorphic DNA (RAPD), rep-PCR, and ERIC-PCR produced strain-level information and were useful in the analysis of the production of probiotics (Gevers et al., 2005). Such early molecular technologies had laid the necessary foundation for later advances with multilocus sequence typing (MLST), digital DDH, average nucleotide identity (ANI), and eventually whole-genome sequencing (WGS) technologies. Phenotype into genotype could now be considered a trend that still applies in the current revision in the 21st century of various probiotic species. Indeed, more than 250 species have been identified in the genus Lactobacillus over the past decades. However, in current revisions led by 15 scientists around the world, the genus Lactobacillus was reorganized into 25 genera, including 23 newly proposed genera, according to Zheng et al. (2020).

3.3. Advancements in Technology for Probiotic Validation

The last two decades have witnessed a tremendous transformation of the probiotic authentication toolset, caused by technological innovations, shifting from a phenotype-description approach towards a high-resolution, genomic-driven toolset. Variations and advances, such as molecular microbiology, specifically PCR, DNA sequencing, and molecular fingerprinting, brought about the first paradigm shift towards feasible probiotic authentication. However, with the advent of species-specific PCR, ARDRA, RFLP, and rep-PCR, there was a tremendous increase in enhanced detection with reduced time, compared to conventional methods, and also accurately discriminated between probiotic and closely related taxa (Giraffa et al., 2000; Gevers et al., 2005). However, with advancements and breakthroughs with Sanger sequencing and later 16S rRNA phylogenetics, there was a radical transformation towards a reproducible probiotic taxonomy (Janda & Abbott, 2007; Salvetti et al., 2012). Later, next-generation sequencing technology, such as WGS, brought about a tremendous change, which is a precise identification of a strain through parameters of average nucleotide identity, digital DDH, and phylogenomics of the core-genome and pan-genome (Chun et al., 2018; Richter & Rosselló-Móra, 2009). Whole genomic sequencing is now accepted and authenticated as a gold standard technique and technology, as it reads the entire DNA sequence of a strain, which can unambiguously distinguish even

extremely closely related strains. For this reason, it is applicable and useful for confirming commercial probiotics, measuring consistency among different batches, and also emphasizing detectable antibiotic-resistant genes (Lugli et al., 2023; Zheng et al., 2020). However, WGS technology brought about a radical change, and a determinant move towards revised taxonomy among the *Lactobacillus* genus, where there is a rewritten position towards 25 genera, and that also till 2020 (Zheng et al., 2020). The advent of shotgun metagenomics and amplicon-based microbiome analysis has made it possible to perform probiotic authentication in even intricate sample matrices like fermented foods, nutritional supplements, and the human gut. This is even more helpful in big strain complexes and non-culturable and slow-growing microbes. Fourth-generation shotgun metagenomics allows strain-level discrimination with lower abundance identification, making possible post-marketing authentication and confirmation in commercial probiotic preparation (Fujimoto et al. 2020; Tonkin-Hill et al. 2022). Another benefit is reduced dependence on appropriate culture-based probiotic identification methods, as they inaccurately determine viable but non-culturable (VBNC) probiotic microbes. Advanced genomic analysis software like KBase, Prokka, GTDB-Tk, CheckM, Roary, and Anvi'o facilitates automated and generic genomic annotation and strain-level comparisons and analysis. Online tools such as dDDH (GGDC) or ANI fast calculation software enable accurate demarcation at or below the species level (Jain et al. 2018; Meier-Kolthoff et al. 2013). The technological advancements have directly influenced the probiotic regulatory and quality assurance framework across the globe.

4. CONTEMPORARY APPROACHES TOWARD IDENTIFICATION OF PROBIOTIC

The concept of bacterial strain definition has undergone a paradigm shift with the advent of new molecular technologies that have the ability to provide detailed and discriminative information regarding the genomic identification of bacteria. The definition of a strain is even suggested by Gevers et al. (2001) and Van Rossum et al. (2020) to have no, or at best a poor, biological basis. This has sparked discussions about the ecological validity and functional meaningfulness of strains as hitherto defined, along with possible gaps when attempting to scale from the laboratory-derived observations into more complex natural ecosystems (Achtman & Wagner, 2008; Amann et al., 1995; Prosser et al., 2007). There is a general consensus that all probiotic effects depend on specific strains and that it is not appropriate to attribute the properties or health effects of one particular strain to other strains within the same species or to the strains of other species (Lee et al., 2013). A systematic review and meta-analyses published in 2018 further emphasize that probiotic efficacy will be highly dependent upon both the specific strain and the health condition in question (McFarland et al., 2018). Again, this emphasizes the need for identification and quantification techniques that have the ability to identify the exact probiotic strain, especially in research and manufacturing settings where product efficacy should be well warranted. There are many traditional culture-based plate count techniques and CFU measurements have remained the gold standard for quantifying viable probiotic bacteria. However, they lack the resolution to measure individual strains in multi-strain formulations. Therefore, manufacturers often use a combination of methods, employing culture-dependent counts for overall viability and genomic tools for confirming identity, often only to the species level (Jackson et al., 2019).

Current methodologies for identification and characterization of probiotic strains are summarized in table1. The use of phenotypic identification methods, such as carbohydrate fermentation patterns, enzymes, and cell shape, only provides a preliminary identification but fails to do so on a strain level (Hutkins et al., 2016). However, the use of molecular biology or genotypic methods has dominated and 16s rRNA gene sequencing identifies the species, but whole genome sequencing or Average Nucleotide Identity offers identification on the strain level (Salvetti & O'Toole, 2017). Polymerase chain reaction-based methods, multiplex PCR, and strain-specific primers are commonly required in quality control checks today (Fukao et al., 2019). MALDI-TOF MS offers rapid protein profiling identification on the species, but can rather require the updating of species data to offer identification on the strain level (Dumolin et al., 2019). Today, the applications within metagenomics and comparative genomics are becoming evident in the role played in the identification, prediction, and search for contaminants (Schlaberg et al., 2017). A flow graphical image of current methods for the identification of a probiotic strain is shown in Figure 2.

5. CURRENT ISSUES IN IDENTIFYING PROBIOTIC STRAINS

The correct classification of probiotic bacteria still represents a basic demand for authenticity, safety evaluation, compliance with regulations, and the possibility of repeatability in clinical trials. But, certain

Table 1: Current Methodology for Strain-Level Characterization of Probiotics

Methods	Techniques	Proficiency	Constraints	References
Phenotype-based methods	Biochemical tests, metabolomics, MALDI-TOF MS	Rapid, cost-effective, functional insights	Low resolution; environment-dependent	Djukovic et al., 2022
Genotypic methods	MLST, PFGE, RAPD, CRISPR typing	Discriminatory for many bacterial species	Labor-intensive; limited genomic coverage	Maiden et al., 2013; Shariat & Dudley, 2014
Whole-genome sequencing (WGS)	Hybrid assemblies (Illumina + Nanopore/PacBio)	Highest resolution, enables gene-level analysis	Data processing demand; cost	Quainoo et al., 2017
Comparative genomics	ANI, SNP profiling, core genome analysis	Accurate strain differentiation, evolutionary insights	Standards vary between databases	Jain et al., 2018; Parks et al., 2020
Metagenomic strain tracking	StrainPhlAn, in Strain, DESMAN	Captures uncultivable strains in complex microbiomes	Computationally intensive, depth-dependent	Albanese & Donati, 2017; Olm et al., 2021
Single-cell sequencing	Microfluidics, Hi-C, long-read cell-omics	Captures heterogeneity and rare strains	High cost, requires advanced platforms	Tian et al., 2020); Jovic et al., 2022

biological, technical, and methodological difficulties persist to make classification even more complex, even with the latest advancements in the field of molecular microbiology. Most probiotic species, especially *Lactobacillus, Bifidobacterium,* and *Bacillus*, harbor extremely similar average nucleotide identity values with percentages of more than 99%, which hampers the classification of each strain with accurate techniques of phenotyping or the use of lower resolution molecular methods. The 16S rRNA gene similarities range from more than 99.5% in the comparative study of the strains, making it impossible to classify the genetic diversities of distinct categories at the strain level, even with the use of whole genome sequencing due to the possibility of differences in the size of the relatively small mobile genetic constituents, such as plasmids or phage genomes of the bacteria only (Pasolli et al., 2020). Other methods of phenotyping include carbohydrate fermentation patterns, cell morphology, and enzymatic assays, with variables of lower reproducibility and extremely high intraspecific divergences between species. Such methods are considered to be highly susceptible to physiological and stressed states in culturing conditions due to physical, chemical, and environmental impacts (Salvetti et al., 2012). As the technique works under the principle of possibly mismatched genotype and phenotype properties, the accuracy of tests performed by this technique cannot be considered reliable to strictly classify the strains to be used in probiotic preparations recommended to consumers by the probiotic industry. The technique of using the 16S-rRNA, groEL, recA, and hsp60 genes separately involves a path of lower sensitivity to genetic diversities with extremely low percentages of the intraspecific genetic divergences of the bacteria.

Probiotic bacteria frequently gain or lose genetic material by the process of Horizontal Gene Transfer, resulting in genetic diversity within the same phylolineage. Mobile genomic elements, plasmids, integrins, and phages can impact characteristics like carbohydrate metabolism, adhesion, antimicrobial resistance, and stress response (Sun et al., 2023; Ye et al., 2023). Metagenomics enables the characterization of strains in complex samples like fermented food and feces, but it has limitations because it utilizes short-read sequencing that fails to distinguish certain repetitive regions within genomes to distinguish strains. However, this has better resolution with long-read metagenomics but is still expensive and

computationally demanding. Lack of universal standards for naming, annotating, and submitting strains leads to incongruous taxonomies, particularly due to changes in Lactobacillaceae (Choi et al., 2023; Zheng et al., 2020). There has been empirical evidence suggesting common misidentification, strain swapping, and contamination of some probiotics available in markets due to inadequate validation during both production and post-market evaluation (Das et al., 2025). Disparity between different regulatory requirements (EU demands WGS but often not in the USA) poses problems for the validation of probiotics' authenticity. This constitutes a problem in comparing studies targeted at validating probiotics across different disciplines because of the disparity between different standards of validation. There are many validation problems associated with probiotics, including high levels of similarity in genomic structures, high levels of flexibility within their genomic structures, and inadequacies in the tools available to execute validation. Advanced tools like hybrid long-read whole-genome sequencing, pan genomics, classifiers based on machine learning, and new frameworks to act as universal references make big steps toward validating authenticity within probiotics, but they have to be harmonized on a universal platform to improve their success rate.

6. CONSTRAINTS OF EXISTING PROBIOTIC IDENTIFICATION METHODS

Identification and enumeration of probiotic microorganisms with accuracy have constituted the core difficulties within the context of quality and regulatory controls as well as those related to research mechanisms. Although many analytical methods have been developed from the classical approach in the field of microbiology to the current genomic and cytometric analytical methods, limitations within the methodology have existed. Culture-based approaches, especially colony-forming unit (CFU) counts using selective agar, also persist owing to official acceptability and long tradition. Nonetheless, CFU counts tend to be lower than the number of living cells because VBNC bacteria cannot be enumerated when such bacteria may be metabolically competent and functionally important even if unable to develop colonies under standard lab conditions. Selective agar also continues to be a major drawback; no medium would be ideal for all probiotics, while the impact of the medium components and the intrinsically complex bacterial physiology could lower the number of culturable bacteria even further, artificially underestimating the number of microbial entities present (Vinderola et al., 2019; Zawistowska-Rojek et al., 2022). Additionally, these methods are time-consuming, operator-specific, and prone to complications from the natural background microbiota when more than one strain or fermented products of probiotics are used (Boyte et al., 2023; Zawistowska-Rojek et al., 2022).

Molecular analyses, including 16S rRNA gene sequencing, species- or strain-specific PCR, and qPCR, are more sensitive and discriminative, but are necessarily blind to the distinction between DNA from viable and dead bacteria (Boyte et al., 2023). This blindness to viability limits their use in regulatory enumeration, where viable cell number is considered the gold standard. Other challenges relate to the degree of constant variability in the copy number of the 16S gene in different species, making abundance estimates more prone to bias, the difficulty to differentiate between closely related species or strains when analyzing the 16S gene; and the susceptibility to bias due to the differential extraction efficiency and primers used in PCR amplification (Dabban et al., 2024). Shotgun metagenomics enables greater discrimination but comes with a high-cost tag and the need for comprehensive genome resources that do not necessarily include all commercial probiotic bacteria. Viability-correcting molecular methods, specifically those involving propidium monoazide (PMA) or ethidium monoazide (EMA) with qPCR, seek to address vitality restraints by differentiating viable from nonviable organisms by exclusion of dyes from cells with impaired cellular envelopes. Nonetheless, there could be discrepancies in cell permeability for dyes depending on cell walls, phase cultures, and product formulation, thereby partly undermining the efficacy of differentiating viable from nonviable cells, specifically for multiple-strain products. Alternatively, RT-qPCR reactions involving RNA targets would offer enhanced specificity toward viable organisms, but RNA degradation and loss could stand as other challenges (Marole et al., 2024).

Analytical approaches based on proteomics and phenomics, MALDI-TOF mass spectrometry, have been able to provide fast and highly discriminative species identification. However, they have been associated with important limitations. These have been highly dependent on the thoroughness of reference spectral libraries, which are also curbed by the absence of reference to probiotics for industry. Strain discriminatory capacities have been largely impaired. Moreover, MALDI-TOF identification requires well-characterized or pure high-biomass cultures of the unknown; more complex or low-biomass cultures may be poorly detected or result in a

low specificity spectrum (Han et al., 2021). On the other hand, flow cytometry (FC) analysis has been found to be a useful methodology for fast viability detection. It has also been able to differentiate between viable, damaged, and nonviable fractions of cultures by means of fluorescent dyes. Finally, strains characterized by membrane integrity or esterase activity have been found to be inconsistent in predicting the functional activity of probiotics. However, the effects of matrix interactions, the difficulties of preparing the samples, and the high costs of the equipment and the required personnel training have always been seen as important hindrances to its more extensive use for the analysis of the industrial laboratory. However, a common problem with these methods is the lack of standardized analytical procedures. There has been evidence based on several surveys that significant discrepancies exist between product packaging claims of probiotics and their actual viable counts or identity, largely influenced by the employed analytical methods or the absence of strict regulatory control (Ghelardi et al., 2023). Collectively, the shortcomings outlined above indicate that no single analytical technique is adequate for strain-level probiotic authentication and viability analysis. Culture-based CFU enumeration coupled with cytometric, proteomic, or alternative molecular technologies is increasing in popularity with regard to providing a well-rounded analytical strategy. Future applications in the enhancement of known databases and technologies centered upon viability will be integral in improving the current state of probiotic authentication.

7. PROBIOTIC IDENTIFICATION FOR RESEARCH AND INDUSTRY

Probiotics identification is the vital key or prerequisite for academic and industrial applications in ensuring the legitimacy, safety, and identity of functional probiotics. The standardization for the strain identification profiling is shown in Table 2. While in academic applications, strain-level identification helps perform replicate experiments, draw legitimate clinical results, and clarify biological meaning in terms of relationships between the host and microbes. Presently, high resolution in genomic analysis with high-quality tools such as whole genome sequencing (WGS), core genome MLST, or pangenomics has empowered scientists with solid platforms capable of making valid distinctions within very similar strains, as well as pinpointing meaningful functional genes involved in legitimate probiotic functions (Brito et al., 2018). On the other hand, while in probiotic industries, legitimate identification has served as an essential prerequisite in terms of adherence to regulations. Presently, concerns in regulatory authorities are increasingly emphasizing genome-based authentic identification in order to avoid instances of misassignment and contamination commonly exhibited in commercial probiotics (Das et al. 2025). More sophisticated tools in genomic analysis, such as digital PCR, long-read sequencing, or specific species identification assays, are currently being incorporated in production lines.

8. FUTURE ASPECT OF PROBIOTIC IDENTIFICATION

Precise probiotic strain identification and classification remain critical for evaluating the authenticity and safety of probiotics. Though the conventional methods based on phenotypic analysis and single gene identification have been causative for the early definition of probiotics, their sensitivity and discriminatory power do not support the classification of genetically related strains. Recent developments in whole-genome sequencing, related to metagenomics and hybrid-genome approaches, have literally revolutionized the identification and verification process for probiotics to accurately classify and differentiate strains (Wick et al., 2023). Resolution has further been improved by the addition of pangenomics analysis to reveal the core and accessory gene pangenomes to determine the related genetic patterns and deduce the strain-specific functions (Brito et al., 2018). Simultaneously emerging developments regarding machine learning and artificial intelligence approaches have presented novel prospects for enhanced genomic identification and classification for predicting specific biomarkers with improved sensitivity and precision (Libbrecht & Noble, 2015). Currently, enhancements related to metagenomics and multi-omics approaches have been projected to provide direct identification for the related strains at the microenvironment of the human gut microbiome by translating genetic patterns to specific functions (Sabih Ur Rehman et al., 2025).

Further advancements and improvements in the genome of this organism, there is a need for harmonization of standards on a global scale, the genomic databases, and fast molecular-based techniques for the authentication of strains directly during production and usage. Technology that will boost efficacy and authenticity includes barcoding techniques that make use of CRISPR/Cas Systems and Digital Fingerprinting Strategies (Gomaa, 2020). The advanced techniques and their applications and

Table 2: Standardization for Probiotic Strain Identification Profiling

Standardization area	Specifications	References
Average nucleotide identity (ANI ≥ 95%)	Species-level inclusion threshold in genomic taxonomy	Richter & Rosselló-Móra, 2009
Genome quality metrics	>90% completeness & <5% contamination benchmarks	Bowers et al., 2017
Fair data principles	Ensure strain genomic data are findable, accessible, interoperable, reusable	Wilkinson et al., 2016
Minimum information about a genome sequence (MIGS)	Required metadata for genome submission	Field et al., 2008

Table 3: Emerging Approaches and Strategic Directions of Probiotic Strain Identification

Advanced Techniques	Applications	Significances	References
Pan genomic analytics	Captures the full gene pool of the species, including strain-specific genes	Enables precision functional prediction	Tettelin & Medini, 2020
AI-driven strain prediction	Identifying strain behavior from sequence signatures	Improves probiotic safety and efficacy forecasting	DeMaere & Darling, 2019
Real-time sequencing in vivo	Portable sequencing for clinical microbiology	Rapid infection control decisions	Quick et al., 2016
Multi-omics integration	Genomics, transcriptomics and metabolomics	Functional validation of strain traits	Hasin et al., 2017
CRISPR-based strain barcoding	Live tracking of engineered or therapeutic strains	Enables precision microbiome interventions	Rubin et al., 2022

significances are summarized in Table 3. On the whole, the future of probiotic strain identification is increasingly becoming a genome-centric approach utilizing AI to ensure a standardized process, which will greatly improve accuracy and confidence in probiotic research and products. A pictorial representation of the potential approaches for probiotic identification is given in Figure 3.

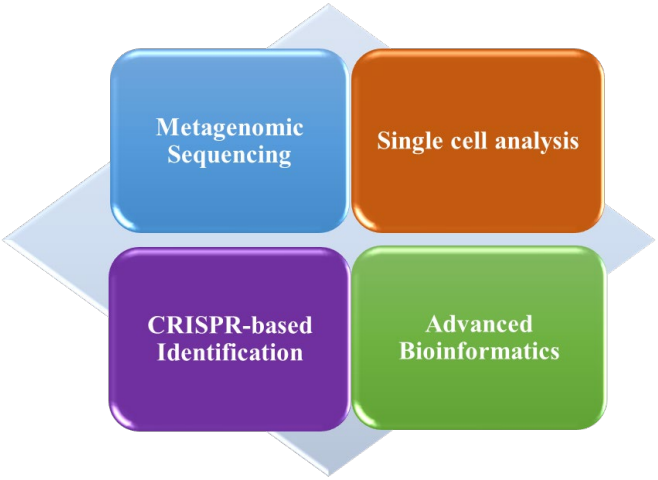

Figure 3: Future Direction of Probiotic Strain Identification

9. CONCLUSION

Probiotic strain identification has also undergone a radical transformation from conventional phenotypic tests towards the use of cutting-edge genomic and bioinformatics approaches. Strain-level verification is crucial for ensuring the efficacy, safety, and reproducibility of probiotics and their regulatory approval. Although conventional phenotypic and genotypic tests are helpful tools for initial strain identification, these tests are inadequate and unspecific for distinguishing between strains that are closely related and mixed strain formulations. Whole-genome sequencing now stands as the most reliable technique and approach with properties and utilities associated with precise strain-level resolution, functional gene and strain annotation, and strain-level strain safety and risk evaluation. Metagenomics, a culture-independent approach, also increases the ability and capacity of whole-genome sequencing and associated analytic tools and approaches towards the detection and quantification of strains within complex biological samples. Application of polyphasic strain identification approaches and strategies, incorporating integration of phenotypic, genotypic, genomic, and metagenomic data, will make up for the shortcomings of these methods and approaches and help provide effective and efficient strain-level verification with reproducibility and compliance with regulatory requirements. However, there have been limitations and challenges, including incompleteness of databases, the requirement of expert technical talent and qualified manpower, and cost and regulatory issues. However, there are promising innovations, including the use of pan-genomics and AI-assisted strain identification as tools and approaches that are poised to have a revolutionary effect and make radical transformations within the context of strain identification within probiotics and associated biotechnology.

List of Abbreviations

AI – Artificial Intelligence
ANI – Average Nucleotide Identity
API – Analytical Profile Index
ARDRA – Amplified Ribosomal DNA Restriction Analysis
CFU – Colony-Forming Unit
DDH – DNA–DNA Hybridization
EFSA – European Food Safety Authority
ERIC-PCR – Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction
FAO – Food and Agriculture Organization
FDA – Food and Drug Administration
HGT – Horizontal Gene Transfer
ISO – International Organization for Standardization
LAB – Lactic Acid Bacteria
MALDI-TOF MS – Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MLST – Multilocus Sequence Typing
PCR – Polymerase Chain Reaction
PFGE – Pulsed-Field Gel Electrophoresis
qPCR – Quantitative Polymerase Chain Reaction
RAPD – Random Amplified Polymorphic DNA
Rep-PCR – Repetitive Element-Based Polymerase Chain Reaction
RFLP – Restriction Fragment Length Polymorphism
RT-qPCR – Reverse Transcription Quantitative Polymerase Chain Reaction
USP – United States Pharmacopeia
VBNC – Viable But Non-Culturable
WGS – Whole Genome Sequencing
WHO – World Health Organization

Acknowledgment

The authors would like to thank the institution for its support for this study.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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Cite this article: Kohinur Begum, Md Shajidur Rahman, Nushrat Jahan Ema. Probiotic Strain Identification: Current and Future Approaches. Asian J. Pharm. Health. Sci. 2026; 16(1):3221-3234. DOI:10.5530/ajphs.2026.16.88