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REVIEW ARTICLE

Functional Molecular Biomarkers in the Identification of Probiotic Strains

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Abstract

Identification of novel probiotic strain candidates based on sequence information of currently available probiotic strains and their key biomarkers requires comprehensive functional evaluation to establish their contributions to human health. Potential functional molecular biomarkers play a vital role in facilitating the discovery of new probiotic strains through sequence similarity analyses, remarkably within metagenomic datasets and unexplored microbial "*dark matter*". It is critical to evaluate probiotic properties beyond taxonomical identifications by including the most important functional, metabolic, antibacterial and immunological features, as well as by using them as a marker for selection of probiotics. This review highlighted some key molecular biomarkers of probiotics, but also considered challenges like lack of standardization and *in vitro* and *in vivo* data, and discussed potential for further use. Probiotic screening by molecular markers in combination with bioinformatic search technology, which might represent future development direction toward the generation of next-generation probiotic strains.

Keywords: Probiotic. Biomarkers. Metabolic. Antimicrobial activity. Gastrointestinal tolerance.

Introduction

The global interest in probiotics has expanded rapidly due to their recognized potential in maintaining health and preventing a wide range of diseases. Probiotics are live microorganisms which provide benefits to a host when administered in sufficient amounts, but the effects of the organisms depend on the specific strain used **[1-3]**. Because of this specificity, researchers cannot accurately predict how well a probiotic works. Nowadays, next-generation sequencing (NGS) technologies have changed this field because of detailed analyses of genomes and metagenomes. In those processes, it is possible for researchers to identify functional molecular biomarkers. To evaluate a microbial strain, the biomarkers are essential tools. As an example, they show whether a strain has the attributes that are necessary to survive conditions in the gastrointestinal tract. And they show that a strain can live in the host to produce physiological effects that are beneficial **[4,5]**.

Continuous scientific and commercial interest in probiotics is also reflected in their rapidly growing global market, driven by their broad range of applications and strong safety profile **[6]**. Probiotics have therapeutic and prophylactic potential against numerous conditions, including hospital-acquired infections, cancer, pancreatitis, necrotizing enterocolitis, *Helicobacter pylori* infections, irritable bowel syndrome, autism spectrum disorder, and various infectious and autoimmune diseases **[6]**. According to the International Scientific Association for Probiotics and Prebiotics, the mechanisms underlying probiotic action include colonization resistance, production of organic acids and short-chain fatty acids, regulation of intestinal transit, normalization of disrupted microbiota, enhancement of intestinal epithelial cell turnover, and competitive exclusion of pathogens **[6]**.

In 2021, the global probiotic market was estimated at approximately US$ 47.6 billion, while the European market reached an estimated € 9.4 billion in 2022, representing a growth of over 9% compared to 2018 (https://ipa-biotics.org/2023-events-overview/). Together, these developments highlight the increasing importance of advanced molecular approaches, particularly biomarker-based evaluation, in guiding the discovery, validation, and application of next-generation probiotic strains **[6,7]**.

This review highlighted some key molecular biomarkers of probiotics, but also considered challenges like lack of standardization and *in vitro* and *in vivo* data, and discussed potential for further use. Probiotic screening by molecular markers in combination with bioinformatic search technology, which might represent future development direction toward the generation of next-generation probiotic strains.

Molecular biomarkers of probiotic functionality

Functional molecular biomarkers, which are biological information in terms of DNA, RNA and protein sequences related to the function and efficiency of the probiotic strains, play a critical role in understanding mechanisms behind probiotic functionality, and connecting *in vitro* results with *in vivo* data. Well-studied functional biomarkers can enable effective selection of probiotic strains and facilitate approval and development of beneficial probiotic products **[8]** (Table 1). Efficient probiotic product selection is closely associated with an array of functional biomarkers that could predict the survival ability, colonizing capacity, and beneficial function of microorganisms. Gastrointestinal survival biomarkers can be considered essential as probiotic organisms must tolerate diverse physiological conditions while passing through the gastrointestinal system, which consists of a gastric compartment containing stomach acids, bile salts, and digestive enzymes in a distal part of the small intestine. Markers related to survival ability are those relating to acid tolerance, bile salt hydrolase and the pancreatic enzyme resistance **[8,9]**.

Table 1. Summary of molecular biomarkers, their strains, function and mechanism.

Biomarker class	***Microbail Strains***	***Mechanisms***	***Function***
Gastrointestinal Survival	*L. rhamnosus GG, B. coagulans* (GBI-30, 6086), *Saccharomyces boulardii, etc*	Acid tolerance, BSH activity	Survival in gut
Adhesion & Colonization	*L. rhamnosus* GG, *L. plantarum* WCFS1, *Bifidobacterium infantis* 35624	Pili, Mucus-Binding Proteins	Colonization
Antimicrobial Activity	*L. plantarum* and *lactis, Bifidobacterium, Enterococcus*, and *B.* species,	Bacteriocins, organic acids	Pathogen inhibition
Immunomodulation	*B. infantis 35624, L. casei Shirota*	Cytokine modulation, TLR interaction	Antiinflammatory
Metabolic Activity	*B. lactis BB-12, L. plantarum*	SCFA production, vitamin synthesis	Gut health
Enzymatic Activity	*L. acidophilus NCFM, Bifidobacterium spp.*	β-galactosidase, proteases	Digestion
Barrier Function	*L. rhamnosus GG, L. plantarum*	Tight junction regulation	Gut integrity

Note: L: *Lactobacillus; B: Bacillus, BSH: Bile salt hydrolase; TLR: Toll-like receptors. SCFA: Short-chain fatty acids.*

Gastrointestinal Survival Biomarkers

Probiotic microorganisms' survival in the gastrointestinal tract is essential for probiotic action since the microorganisms should survive gastric acidity, bile salts and enzymes in the digestive system in order to survive and multiply in the intestine. Some well-documented strains show resistance to low pH and bile environment. For example, *Lactobacillus rhamnosus* GG (ATCC 53103) show the highest resistance to the low pH and bile due to the presence of *GroEL, DnaK* stress-response systems **[10]**. Similar mechanism of stress resistance, including bile salt hydrolase (BSH) activity and membrane adaptive systems is observed in *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis* subsp. Lactis BB-12. For spore-forming bacteria like *Bacillus coagulans* GBI-30, 6086, endospore formation may lead to significant improvement in resistance against gastrointestinal stresses **[11]**.

Adhesion and Colonization Biomarkers

Probiotics successful and sustained functionality depends on probiotic adherence to host intestinal surfaces and their capacity to colonize temporarily in the intestinal tract. *Lactobacillus rhamnosus* GG serve as the prototype in relation to adhesion capacity and temporary intestinal colonization **[12]**. SpaCBA pili on

L. Rhamnosus GG are important in mediating strong binding interactions with the mucus and host epithelial cells. Several genes related to adhesion in *L. Plantarum* WCFS1, like mapA and mucus-binding proteins have been identified which facilitate epithelial adherence. Efficient adhesion has been reported for *Bifidobacterium longum* subsp. Infantis 35624 mediated by its production of EPS and surfaceassociated proteins **[12-14]**. Generally, these probiotic strains exhibit highly hydrophobic nature and have strong capability to auto-aggregate and co-aggregate, which are common in vitro indicators of colonization ability.

Antimicrobial Activity Biomarkers

Antimicrobial activities of probiotics play a crucial role in modulating the gut microbial ecosystem and suppressing pathogens. Plantaricins, bacteriocins that exhibit activity towards pathogens such as *Salmonella* and *Listeria*, are produced by *Lactobacillus plantarum* strains WCFS1 and 299v. Nisin, an established bacteriocin, is produced by *Lactococcus lactis,* and its use is prevalent in food processing. Organic acid production contributes to the inhibition of pathogens by Bifidobacterium bifidum, while reuterin produced by *Lactobacillus reuteri* DSM 17938 is an active antimicrobial substance **[15,16]**. The inhibition zones assay, co-culture experiments and detection of bacteriocin gene are commonly used methods to measure such activity.

Immunomodulatory Biomarkers

Immunomodulation plays a vital role in many systemic and local beneficial effects attributed to probiotics. *Bifidobacterium longum* subsp. Infantis 35624 has known antiinflammatory properties, such as increases in IL-10 levels and inhibition of pro-inflammatory cytokine levels (e.g., TNF-). *Lactobacillus casei* Shirota is known to promote innate immunity, with improvements in NK cell activity and alterations in cytokine profiles. *Lactobacillus rhamnosus* GG binds to TLRs, triggering various intracellular signalling pathways and inducing sIgA secretion **[3,17,18]**. These markers represent crucial determinants of the effect probiotics have on immune homeostasis and inflammatory conditions.

Metabolic Biomarkers

The function of probiotics is significant in metabolic aspects in host. Among those, probiotics contribute to the production of SCFAs. SCFAs have key roles to gut homeostasis as well as host metabolism. Some of the strains of probiotics, e.g., *Bifidobacterium animalis* subsp. Lactis BB-12, *Bifidobacterium longum*, is dominant producer of acetic acid. While some strains like Lactobacillus plantarum produce lactic acid, it would be further utilized by other members of microbiota to produce butyric acid **[18,19]**. However, *Faecalibacterium prausnitzii*, which cannot be a conventional probiotic, is an important butyric acid producer linked with anti-inflammatory activity. Also, some probiotics like *Lactobacillus reuteri and Lactobacillus fermentum*, synthesise vitamins such as vitamins B group, which is important in nutrition of host **[20,21]**.

Enzymatic Activity Biomarkers

Enzymatic abilities are vital for probiotic food supplements to enhance digestion and enhance nutrient availability. For instance, *Lactobacillus acidophilus* NCFM exhibits activity of β-galactosidase which can be involved in lactose hydrolysis in addition to alleviating lactose intolerance. Glycosidases are also secreted by *Bifidobacterium breve* and *Bifidobacterium longum*, playing an important role in carbohydrate metabolism. In addition, proteases and phytases are secreted by *Lactobacillus plantarum* to aid protein hydrolysis and release minerals from plant matrices **[22,23]**. These abilities can be measured by means of various biochemical methods.

Intestinal Barrier Function Biomarkers

A key health-promoting effect of probiotics is the maintenance of the intestinal barrier function. *Lactobacillus rhamnosus* GG has been demonstrated to up-regulate the expression of tight junction proteins, including occludin and ZO-1, which in turn increases epithelial barrier function and decrease permeability. *Lactobacillus plantarum* WCFS1 can enhance tight junctions and stimulate mucin secretion, including the MUC2 gene. *Bifidobacterium longum* subsp. Infantis 35624 maintains the integrity of the barrier by attenuating inflammation and modulate the immune system response **[24,25]**. These phenomena can be measured by assessing transepithelial electrical resistance (TEER) and permeability.

Challenges and Limitations

Despite significant advances in the identification and characterisation of functional biomarkers for probiotic microorganisms, several challenges and limitations remain that can affect the reliability and translational relevance of findings. One major limitation is that *in vitro* results often do not accurately reflect *in vivo* conditions. Laboratory assays are typically conducted under controlled and simplified environments that fail to replicate the complexity of the gastrointestinal tract, including dynamic pH changes, host interactions, microbiota competition, and immune responses [26,27]. As a result, probiotic strains that perform well *in vitro* may not exhibit the same efficacy within the human body. Another important limitation is that the presence of specific genes does not necessarily guarantee their expression or functional activity. Genomic analyses frequently identify genes associated with desirable probiotic traits, such as bacteriocin production or adhesion factors; however, gene expression is highly dependent on environmental conditions and regulatory mechanisms. Therefore, functional validation at the transcriptomic and proteomic levels is essential but not always performed [27,28].

Additionally, the lack of standardised protocols across studies poses a significant challenge. Variability in experimental methods, such as differences in simulated gastric models, cell lines used for adhesion assays, or biomarker measurement techniques, makes it difficult to compare results across studies and draw consistent conclusions. This inconsistency limits reproducibility and slows the development of universally accepted criteria for probiotic evaluation [29]. Finally, host variability plays a crucial role in determining probiotic performance. Factors such as age, diet, genetics, health status, and existing gut microbiota composition can significantly influence how a probiotic strain behaves and its overall efficacy. This inter-individual variability complicates the generalisation of results and highlights the need for personalised approaches in probiotic research and application.

Future Perspectives

Bioinformatics tools and innovative approaches will accelerate future developments and research in probiotics by incorporating new sequence information technologies. In terms of the future direction of the technology, the most promising aspect is multi-omics data integration [28]. The inclusion of whole-genome sequenced information, transcriptomics, proteomics and metabolomics, in future research, along with AI will give more efficient information regarding the functionality of probiotic bacteria, and will move researchers away from simple biomarker detection toward understanding interaction between microbe's genes, the expressed protein products, and the metabolite production [27-29]. Integration of these data, including analysis of interactions in the host's response to these microbial features in healthy and diseased status and detection of the function-relevant microbes, would then be able to promote a targeted use of probiotic microorganisms in the future. In the future, both the usage of artificial intelligence and machine learning are predicted to be used to enable probiotic discovery and biomarker prediction. Artificial intelligence can be employed to screen large amounts of data from multipleomics experiments and analyse the patterns to predict and determine functional features such as the antimicrobial and immunomodulatory effects of probiotic microbes. It can further be used to increase strain screening rate, optimisation of probiotic formulation and to improve accurate prediction of probiotic efficacies that require laborious work [29-31]. Finally, personalized probiotics will be developed to suit individual microbiome compositions; as different people have variations in their gut microbes and a single microorganism might not be beneficial for everyone, future research will focus on selecting or engineering strains according to individual microbiomes and factors like genetics and diet for personalized therapy and improved clinical outcomes, especially for chronic diseases.

Conclusion

Molecular markers are not only important for taxonomy and characterisation of microbial strains, but also critical for screening and verification of novel probiotic candidates. Molecular markers such as those based on sequence homology help in the identification of new strains and provide essential information for various functional characteristics, like survival and colonization in the GI tract, antimicrobial effects, immunomodulation, and metabolism of microorganisms. Linking the genomic sequence data to functional markers along with multiomics technologies will lead to the development of next-generation probiotics. Linking of the microbial strains to tangible biological activities can also improve the reliability, reproducibility and confidence of the selection process for clinical and nutritional uses.

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Similarity Check

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Application of Artificial Intelligence (AI)

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