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Probiotics: Characteristics and Screening Methods

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❖ **Introduction:** Probiotics represent a group of live, health-promoting microorganisms that colonize in body and play a fundamental role in maintaining physiological homeostasis (Ranjha et al., 2021). Probiotic organisms, particularly species of *Lactobacillus, Bifidobacterium* and certain beneficial bacteria, help restore this balance by enhancing beneficial microbial populations, suppressing harmful pathogens, and promoting mucosal immunity.

❖ **Mode of action of probiotics** (Nguyen et al., 2022):

1. **Competition with pathogens for space and nutrients:** Beneficial bacteria hinder the colonisation of harmful pathogens by inhibiting microbial adhesion sites competitively.
2. **Production of substances that inhibit pathogen growth:** Antimicrobial organic acids, bacteriocins, hydrogen peroxide and free fatty acids are produced by probiotic microbes. Probiotics inhibit pathogen growth by stimulating the host's synthesis of β-defensin and IgA.
3. **Stimulation of nonspecific immunity:** Different probiotics administered orally have demonstrated adjuvant-like effects on intestinal and systemic immunity such as improved phagocytic activity and enhanced immunoglobulin A (IgA). By maintaining tight junctions and promoting mucin synthesis, probiotics also provide gut barrier.

❖ **Characteristics of probiotic bacteria:** On the basis of the current understanding of these interactions, there are a number of features that are important considerations for strain selection (Shi et al., 2016).

☞ Table 1. Properties of probiotic bacteria given on next page.

❖ **Methods of probiotic screening:**

Indian Council of Medical Research (ICMR) and Department of Biotechnology (DBT) formulated relevant guidelines for use in evaluating probiotics. These guidelines were approved for use in India. The guidelines define the parameters required for a product or strain to be termed 'probiotic.' Probiotic studies generally involve three major components: determining their survival in the

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Table 1. Properties of probiotic bacteria	
Properties	**Description**
Surviving stress within the host	Tolerance to the harsh acidic, enzymes and bile conditions of the GIT is vital for the selection of probiotic microorganisms.
Adhesion to Intestinal Cells	Probiotic organisms should be able to attach onto the intestinal epithelium. This ability prevents the peristaltic movements of the digestive tract from removing the organisms before they confer their benefits. Adhesion and colonisation of probiotics to the mucosal surfaces inhibit pathogens through competition for binding sites and nutrients.
Antimicrobial activity	Probiotic bacteria inhibit pathogens by producing organic acids, hydrogen peroxide, alcohols and bacteriocins.
Antibiotic susceptibility	It is important that probiotic organisms are not inhibited by antibiotics, because strains with intrinsic antibiotic resistance could in fact be useful for restoring gut microbiota after antibiotic therapy. It is important that probiotic bacteria does not carry transferable antibiotic-resistance genes.
Production of biogenic amines	Biogenic amines are organic compounds produced in foods due to the decarboxylase activity of microorganisms as a defence mechanism against acidic environments. Ingestion of foods containing high levels of biogenic amines is associated with vasoactivity and psychoactivity. Some biogenic amines are also potential precursors of nitrosamines when nitrosatable agents are present in the food. It is thus important that a probiotic organism does not produce biogenic amines in foods.
Free from virulence factors	It should also be free from virulence factors, such as toxin genes, hemolysins, or invasins.
Immunomodulatory effects	Probiotics play a significant role in regulating the immune system. They stimulate the production of secretory IgA, enhance the activity of macrophages and dendritic cells, and modulate T-cell responses. Some strains help reduce inflammation by lowering pro-inflammatory cytokines (e.g., TNF-α, IL-6) and increasing anti-inflammatory ones (e.g., IL-10).
Technological Properties for Industrial Use	For use in foods and supplements, probiotic strains must also possess good technological properties, such as: • Stability during processing, fermentation, and storage • Tolerance to freeze-drying or spray-drying

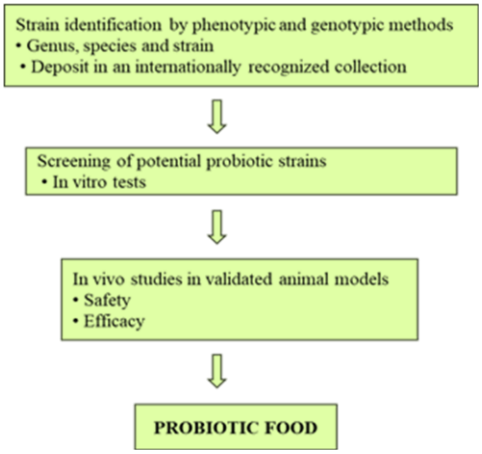

Fig. 1 Probiotic screening process (Byakika et al., 2019)

GIT, safety for human or animal use, and establishing probiotic activity/benefit to the consumer.

❖ Conclusion

Probiotics are valuable biological agents that support gut health and immunity through several complementary mechanisms. Effective probiotic strains must not only survive the gastrointestinal environment but also adhere, colonize, and interact safely with

Table 2. *In vitro* assays employed during screening for novel probiotic strains (Papadimitriou et al., 2015).

Probiotic property	Assays
Surviving stress within the host	• Low pH and bile (e.g., artificial gastric and pancreatic juices and GIT simulators) • Phenol tolerance • Lysozyme tolerance
Safety assays	• Hemolytic activity • Production of toxins (e.g., cytolysins) • Production of enzymes (e.g., glycosidases) • Antibiotic resistance • Production of biogenic amine
Colonization of the host	• Cell surface hydrophobicity • Adhesion to mucus (e.g., adhesion to mucin, enzymatic activity of GAPDH) • Adhesion to intestinal epithelium (e.g., cell-lines, tissue fragments and whole tissue models) • Auto-aggregation screening
Antimicrobial assays	• Production of antimicrobial metabolites such as organic acids and bacteriocins (e.g., bioluminescence assay, simple inhibition tests, turbidometric assays, streak methods) • Co-aggregation with pathogens • Enhancement of intestinal barrier function (e.g., TER measurement, immunofluorescence of tight junction protein antibodies, tight junctional protein phosphorylation)
Probiotic activity test	• β-Galactosidase activity • Total Antioxidative Activity (TAA) • Anticancer activity
Immunomodulation	• Bacterial translocation in the GIT • Co-culture models mimicking in vivo situation • Interaction of host immune system with bacterial compounds (e.g., lipoteichoic acids and peptidoglycan)

the host to provide measurable benefits. Modern screening approaches, including stress-tolerance tests, safety evaluations, adhesion studies, and antimicrobial assays, enable researchers to identify strains with genuine functional potential. By combining these in vitro assessments with established national guidelines, only microorganisms that are scientifically validated and safe are selected for use. Thus, probiotic research continues to provide reliable candidates for improving health, preventing infections and supporting therapeutic applications.

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