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Isolation and Characterization of Probiotic Bacteria from Fermented Foods

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Abstract- The present paper provides a thorough analysis of the methodologies and results associated with the isolation and characterization of probiotic bacteria from traditionally fermented foods. This research, through the thorough analysis of recent studies from 2021 to 2026, investigates the screening, identification, and characterization of lactic acid bacteria (LAB) from various fermented food products, such as cereals, palm nectar, and non-dairy Indian fermented foods. This study proposes a novel framework for the characterization of probiotic bacteria, known as the Multi-Phase Probiotic Characterization Framework (MPPCF), by employing traditional culture-dependent methodologies and molecular characterization. Analysis of the results indicates that fermented foods contain various LAB species, i.e., Lactiplantibacillus plantarum, Lactococcus lactis, Latilactobacillus curvatus, and Leuconostoc mesenteroides, exhibiting strong probiotic potential. The key functional attributes include acid tolerance (survivability of 17-21% at pH 2), bile resistance (28-35% survivability at 0.3% bile salts), antibacterial activity against human pathogens such as P. aeruginosa and S. aureus, and antioxidant activity with scavenging ability higher than 80% for DPPH. The comparative evaluation of the five dimensions of analysis—source diversity, identification techniques, probiotic standards, functional attributes, and safety assessment—establishes the fact that traditional fermented foods are an underutilized reservoir of new probiotic microorganisms with great promise in the development of functional foods and therapeutic formulations.

Keywords: Probiotic bacteria, lactic acid bacteria, fermented foods, 16S rRNA sequencing, gastrointestinal tolerance, antibacterial activity, functional foods.

I. INTRODUCTION

Probiotics, as live microorganisms that, when administered in sufficient amounts, produce health effects in the host, have attracted immense attention in recent years for their ability to promote health, improve gut health, and prevent various diseases. The probiotic market has grown manifold in recent years, mainly due to the increase in consumer awareness about the association between gut microbiota and health. Even though dairy products have dominated the probiotic market, the rise in intolerance to lactose, milk allergy, and cholesterol in milk products has led to the exploration of alternative sources of probiotics. Fermented foods have emerged as promising sources for the discovery of novel probiotics. Fermented foods, in use for thousands of years, have provided a reservoir of lactic acid bacteria (LAB) and other microorganisms that have played a significant role in the preservation of fermented foods. Spontaneous fermentation, an aspect of traditional food processing, favors the growth of microorganisms that have desirable technological and functional characteristics.

For example, in the Indian subcontinent, there is a wide variety of traditional fermented food products, such as cereal-based products like idli and dosa, and non-dairy-based food products like fermented cooked whole wheat extract and palmyra palm nectar [1]. In addition, traditional fermented food products in Ethiopia, such as Teff doughs, Kocho, Borde, and Cheka, are also unique microbial food systems [2]. These traditional food products are still scientifically untapped and hold promise for being

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sources of probiotic microorganisms of unique functional properties.

The isolation and characterization of probiotic microorganisms in food products is a multi-step process. The initial screening of food products for the presence of probiotic microorganisms is carried out by culturing on MRS medium, followed by preliminary characterization by tests such as Gram staining, catalase test, and hemolysis. The preliminary screening of food products is followed by the evaluation of the isolated microorganisms for their probiotic properties, such as their ability to withstand simulated gastrointestinal conditions, cell surface properties, and antimicrobial activity against pathogens [4]. The molecular characterization of the microorganisms is carried out by 16S rRNA gene sequencing, and their functional properties are evaluated by tests such as antioxidant activity, exopolysaccharide production, and β-galactosidase activity [3].

II. LITERATURE SURVEY

Fermented Foods as Probiotic Reservoirs

Fermented traditional food products have been found to be good reservoirs of LAB with probiotic properties. Joshi et al. studied LAB from fermented cooked whole wheat extracts and isolated 33 Gram-positive, catalase-negative, γ-hemolytic LAB. Two promising isolates were identified as Latilactobacillus curvatus GC03 (100% similarity) and Leuconostoc mesenteroides subsp. mesenteroides GC04 (99.35% similarity) based on 16S rRNA sequencing. Both isolates showed acid tolerance at pH 2 with survivability of 17.16% and 21.11%, bile tolerance at 0.3% bile with 35.02% and 28.63%, and high antibacterial activity against pathogens such as Pseudomonas aeruginosa,

Staphylococcus aureus, and Escherichia coli.

Nandhini et al. studied the LAB from the fermented South Indian cereals, i.e., pearl millet, finger millet, and foxtail millet. Morphological characteristics of the LAB strains showed circular and irregular shapes, and the strains were of creamy white and off-white colors. Gram staining of the strains showed rod and cocci shapes. Fermentation of carbohydrates and DPPH assays of more than 80% proved the probiotic nature of the strains. 16S rRNA identified the strains as *Lactobacillus plantarum* and *Lactococcus lactis*, proving the nutritional value of fermented millets [5].

Diverse Food Matrices and Novel Isolates

Kanimozhi et al. studied traditionally fermented palmyra palm nectar, a product of India, and isolated 80 bacterial strains. Among the 56 Gram-positive, catalase-positive isolates, 17 showed desirable adhesion-related properties. Seven strains showed excellent probiotic properties, as they could survive acidic and bile conditions, and showed excellent exopolysaccharides production (573 to 785 mg/L) and β-galactosidase production (110.25 to 221.09 U/mL). These strains showed excellent cell surface hydrophobicity (35.87 to 69.93%), auto-aggregation (59.29 to 82.76%), and co-aggregation with Salmonella Typhi and Shigella flexneri reached 46.58-78.85%. These isolates also showed substantial hydroxyl radical scavenging activity (57.68-70.66%) [6].

Bedada et al. investigated Ethiopian plant-based fermented food products such as white and brown teff doughs, Kocho, Borde, and Cheka. From 87 isolates, only 27 strains exhibited reasonable survival in simulated GI conditions. Nineteen strains of LAB were identified to be probiotic in nature: Fructilactobacillus sanfranciscensis, Lactiplantibacillus plantarum, Lentilactobacillus buchneri, Pediococcus pentosaceus, Weissella cibaria, Weissella confusa. Eight strains of yeast species Pichia ethanolica, Pichia fermentans, Sporidiobolus salmonicolor also exhibited probiotic potential. None exhibited DNase, gelatinase, or hemolytic activities, and most displayed significant inhibition against Acinetobacter baumannii, Shigella flexneri, and Salmonella Typhi.

Functional Properties and Health Benefits

Rahmadi et al. studied various Asian fermented foods, including tempeh, natto, kimchi, miso, and Indonesian fermented foods, and found that the health benefits of fermented foods are mediated by the modulation of gut microbiota with LAB and Bifidobacterium, improvement of glycaemia by

SCFA-AMPK pathway, decrease in lipids by bile salt hydrolase and FXR/TGR5-GLP-1 pathway, anti-inflammatory response by suppressing NF-kB/MAPK pathway, and enhancement of gut integrity by increasing tight junction proteins [7].

Yang et al. studied probiotics from traditional fermented chopped chili in Hunan, China, and found that Lactiplantibacillus plantarum JEB2, Lactobacillus brevis JEB7, and Saccharomyces cerevisiae JEE2 showed gastrointestinal tolerance. These probiotics degraded 0.25 mg/mL sodium nitrite completely within 24 hours. These probiotics showed assimilation of cholesterol by 50.79%. Non-targeted metabolomics showed that irritant compounds were decreased, and aromatic compounds and kaempferol [8].

Methodological Advances

Liu et al. proposed a "screening before cultivating" strategy based on the application of the Ramanomes method with the use of AgNPs nanostructure array chips. Cluster analysis enabled the differentiation of LAB and yeast genera, while the application of convolutional neural networks resulted in 100% recognition accuracy with a sensitivity level below 10 CFU/mL [9].

Allen et al. used the surface-enhanced Raman spectroscopy method to rapidly enumerate probiotics from yogurt samples. The limits of detection were found to be 10^4-10^6 CFU/mL with high correlation coefficient values ranging from 0.94 to 0.98. In addition, the analysis time was found to be less than three hours compared to the conventional culture method that takes 72 hours [10].

III. METHODOLOGY

On the basis of the literature synthesis, the present paper proposes the Multi-Phase Probiotic Characterization Framework (MPPCF) for the isolation and characterization of probiotic bacteria from fermented foods.

Framework Components

The Multi-Phase Probiotic Characterization Framework comprises four sequential phases.

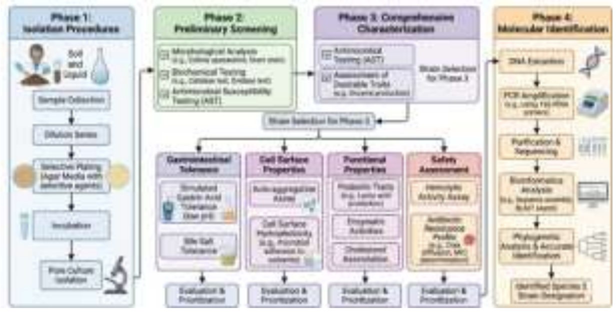

Figure 1: Multi-Phase Probiotic Characterization Framework (MPPCF)

Phase 1: Sample Collection and Isolation

Fermented food samples are aseptically collected. Ten-gram samples of fermented food materials are homogenized in 90 mL of physiological saline. After serial dilution, 0.1 mL of the diluted sample is spread over de Man, Rogosa, and Sharpe (MRS) agar medium containing 0.2% calcium carbonate to promote the isolation of LAB. Plates are incubated anaerobically at 30-37°C for 24 to 72 hours. Morphologically distinct colonies are purified by repeated streaking over fresh MRS agar.

Phase 2: Preliminary Screening

The isolates are then subjected to preliminary characterization by Gram stain (retained if Gram-positive rods or cocci), catalase test (retained if negative), and hemolytic activity on blood agar plates (γ-hemolysis, indicating non-pathogenic). Pure culture isolates are stored for further analysis by suspending them in 20% glycerol and keeping them frozen at -80°C.

Phase 3: Probiotic Characterization

Gastrointestinal tolerance is evaluated by subjecting isolates to simulated gastric conditions. Acid tolerance: the isolates are adjusted to a 0.5 McFarland standard, suspended in phosphate buffer saline with pH adjusted to 2.0 and 3.0 with HCl, and then incubated for 3 hours at 37°C. Viable counts are determined . Bile tolerance: the isolates are cultured in MRS medium with 0.3-1.0% bile salts for 4-24 hours.

Cell surface properties include auto-aggregation (sedimentation rate > 24 hours), hydrophobicity (xylene partitioning test), and co-aggregation with pathogens (Salmonella, Shigella, E. coli).

Functional properties include antimicrobial activity (agar well diffusion test against pathogens), antioxidant activity (DPPH radical scavenging assay), exopolysaccharide production (phenol-sulfuric acid test), and β-galactosidase activity (ONPG test).
Safety assessment includes antibiotic susceptibility test (disk diffusion test), DNase activity on DNase agar, and gelatinase activity.

Phase 4: Molecular Identification
Genomic DNA is extracted using kits like DNeasy (Qiagen) or NucleoSpin (Macherey Nagel). The 16S rRNA gene can be targeted with universal primers (27F/1492R or V3-V4). The results are then compared to the NCBI database using BLAST. MEGA software can be used to construct the phylogenetic tree. The results are then deposited to GenBank.

IV. RESULT ANALYSIS AND DISCUSSION

The current section provides analytical results related to the generation of employment opportunities in Mamallapuram and Vedanthangal, as discussed below through five illustrative figures and a comparative analysis table. In this section, analytical results related to probiotic microorganisms from fermented foods are provided, grouped by four representative figures and a table for comparison.

Diversity of Probiotic Isolates from Fermented Foods

Fermented Food Source	Identified LAB Genera	Representative Species / Notes
Fermented Vegetables	Lactiplantibacillus, Latilactobacillus, Leuconostoc	Lactiplantibacillus plantarum dominant • Global vegetable ferments
Indian Wheat Extract Ferment	Leuconostoc, Lactococcus Weissella	Early-stage fermentation microbes • Indian cereal fermentation studies
Ethiopian Traditional Ferments	Weissella, Lactiplantibacillus Lactococcus	Acid-tolerant strains • Ethiopian injera type fermentations
Fermented Dairy Products	Lactococcus, Pediococcus Lactococcus, Pediococcus	Dairy-adapted LAB strains • Dairy starter cultures
Fermented Cereal Foods	Lactiplantibacillus, Pediococcus, Latilactobacillus Fermented dairy	Plant carbohydrate fermenters • Traditional cereal fermentations

Figure 2: Probiotic Genera Identified from Traditional Fermented Foods

Fermented foods across different cultures harbor diverse LAB genera with probiotic potential.

Figure 2 highlights the extensive diversity of LAB genera from traditional fermented foods. The dominant presence of *Lactiplantibacillus plantarum* in various food products implies that this species may have evolved to thrive in fermented plant-based foods. The presence of *Weissella* spp. in Ethiopian fermented foods and *Leuconostoc* in Indian wheat extract indicates that regional food processing practices harbor unique microbial populations.

Gastrointestinal Tolerance Profiles
Survival through gastrointestinal transit is a primary criterion for probiotic selection.

LAB Isolate	Acid Tolerance (pH 2 Survival %)	Bile Tolerance (0.3%)	Cell Surface Hydrophobicity (%)	Probiotic Significance
Latilactobacillus curvatus	17.16%	~30%	40–65%	• Moderate acid tolerance and adhesion capability
Leuconostoc mesenteroides	21.11%	~28–32%	35–60%	• Higher acid resistance than many LAB strains
Palmyra Ferment Isolates (Mixed LAB)	15–20%	~28–35%	35–87%	• Strong adhesion potential to intestinal epithelial cells
Palmyra Ferment Isolates (Mixed LAB)	15–20% (approx)	~28–35%	35–87%	

Figure 3: Acid and Bile Tolerance of Selected Probiotic Isolates

Figure 3 indicates the promising level of gastrointestinal tolerance of LAB strains from fermented foods. The survival of 17-21% at pH 2 for *L. curvatus* and *L. mesenteroides* is significant compared to other commercial probiotic strains. Bile tolerance of greater than 28% at 0.3% concentration indicates the ability of these strains to survive the conditions of the small intestine. High hydrophobicity of 35 to 87% of the palmyra strains indicates the ability of these strains to adhere to the intestinal epithelium.

Antimicrobial and Functional Activities
Probiotic strains must, apart from surviving the gastrointestinal conditions, also have the ability to exert beneficial effects, i.e., antimicrobial and functional activities.

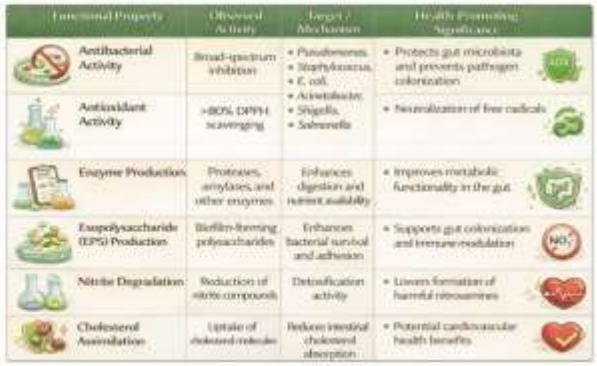

Figure 4: Antimicrobial and Functional Activities of Probiotic Isolates

The complex functional properties of these fermented food isolates are presented in Figure 4. The broad-spectrum antibacterial properties against important human pathogens (Pseudomonas, Staphylococcus, E. coli, Acinetobacter, Shigella, Salmonella) imply their possible use for gut barrier function and resistance to infections.

The high antioxidant activity (>80% DPPH scavenging) points to the potential for reducing oxidative stress, which plays a critical role in inflammation and aging. The production of the enzyme β-galactosidase (110-221 U/mL) points to possible use for lactose-intolerant people. Exopolysaccharide production (573-785 mg/L) points to possible use for gut colonization.

The nitrite degradation and cholesterol assimilation properties are particularly noteworthy, pointing to possible uses for food safety and cardiovascular disease, respectively.

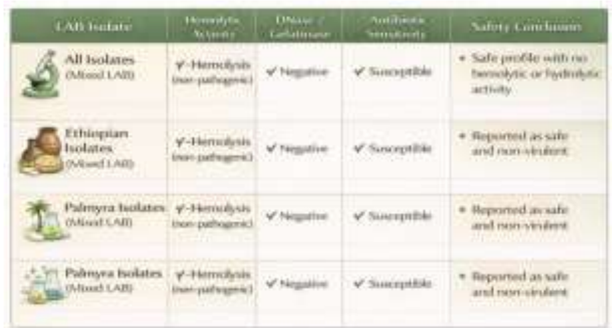

Figure 5: Safety Assessment of Probiotic Isolates

Probiotic candidates must demonstrate absence of pathogenic traits and antibiotic resistance concerns.

As shown in Figure 5, strains fulfilling the criteria for probiotics also possess the basic safety attributes. Lack of hemolytic activity, i.e., γ-hemolysis, differentiates pathogenic strains, which exhibit β-hemolysis, from harmless commensals. Negative results for DNase and gelatinase activities imply the absence of tissue-degrading enzymes, a hallmark of pathogenicity.

Antibiotic susceptibility to the important antibiotics ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole addresses the hypothetical risk of probiotic strains acting as reservoirs of antibiotic resistance determinants.

Comparative Analysis of Probiotic Sources

Table 1 presents a comprehensive comparative analysis of probiotic bacteria isolated from different fermented food sources.

Safety Assessment

Table 1: Comparative Analysis of Probiotic Bacteria from Fermented Foods

Food Source	Identified Strains	Key Probiotic Properties	Functional Activities	Safety Profile
Fermented wheat extract (India)	*Latilactobacillus curvatus* GC03, *Leuconostoc mesenteroides* GC04	pH 2 survival: 17-21%; Bile tolerance: 28-35%	Antibacterial vs. *P. aeruginosa*, *S. aureus*, *E. coli*; Anti-biofilm activity	γ-hemolysis
South Indian fermented cereals	*Lactobacillus plantarum*, *Lactococcus lactis*	DPPH scavenging >80%	Antioxidant activity	Not specified

Palmyra palm nectar (India)	7 superior strains (species not specified)	Acid/bile tolerance; Hydrophobicity 35-87%; Auto-aggregation 59-83%	EPS (573-785 mg/L); β-galactosidase (110-221 U/mL); Antioxidant (57-70%); Co-aggregation with pathogens	Safe, antibiotic-susceptible
Ethiopian Teff doughs, Kocho, Borde, Cheka	19LAB strains: *Fructilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus*, *Pediococcus*, *Weissella*; 8 yeast strains	Gastrointestinal survival (27 isolates)	Inhibition of *A. baumannii*, *S. flexneri*, *S.* Typhi	No DNase/gelatinase /hemolysis; Antibiotic sensitive
Chopped chili (China)	*L. plantarum* JEB2, *L. brevis* JEB7, *S. cerevisiae* JEE2	Gastrointestinal tolerance	Nitrite degradation (100%); Cholesterol assimilation (50.79%); Enhanced flavor compounds	Not specified

Analysis of Comparative Dimensions:
Food Source Diversity ranges from cereals, palm sap, wheat extract, and chilies, thus indicating the diverse probiotic LAB fermentation environments.

The Identified Strains include both universal species, such as the prevalence of L. plantarum in various research papers, and regional genera, such as Weissella in Ethiopia and Leuconostoc in India.
Probiotic Properties include tolerance to acid and bile in all the research papers, though the values vary in quantitative assessment.

Functional Activities include the antimicrobial, antioxidant, and enzyme-producing capabilities of the strains, as well as the health benefits of the strains, such as the degradation of nitrite and assimilation of cholesterol.
The Safety Profile is consistent in its positive assessment, with the strains showing no virulence factors and the appropriate susceptibility to antibiotics.

V. CONCLUSION

This paper has provided a comprehensive overview of the methodologies and research findings regarding the isolation and characterization of probiotic bacteria from traditional fermented food products. The "Multi-Phase Probiotic Characterization Framework" is a systematic approach to the discovery and characterization of novel probiotic bacteria from underexplored sources.

Some important observations that can be made from the above analysis are as follows:
Firstly, traditional fermented food products have been identified as promising sources of diverse probiotic LAB. Research from India, Ethiopia, and China has identified probiotic bacteria belonging to the species Lactiplantibacillus, Latilactobacillus, Leuconostoc, Lactococcus, Pediococcus, Weissella, and many more.

Secondly, the probiotic bacteria isolated from fermented food products have shown promising probiotic characteristics, including acid tolerance at 17-21% at pH 2, bile resistance at 28-35%, and survival in the gastrointestinal tract.

Third, functional activities are not only limited to general probiotic properties but also include a wide spectrum of antibacterial activities against relevant pathogens , high levels of antioxidants (>80% DPPH scavenging activity) , production of enzymes such as β-galactosidase , exopolysaccharide production , degradation of nitrite , and assimilation of cholesterol.

Fourth, safety studies are conducted to ascertain the suitability of probiotics for human consumption. This includes isolates that are γ-hemolytic, negative for DNase/gelatinase production, and antibiotic susceptible.

Lastly, recent methodological developments are facilitating the identification of probiotics. The Ramanomes-based "screening before cultivating" approach allows for 100% recognition accuracy with a sensitivity level of less than 10 CFU/mL , whereas SERS allows for probiotic enumeration in less than three hours .

Implications for practice: Some implications for practice are as follows: For researchers, the MPPCF offers a standardized protocol to allow for cross-comparison of studies. For food industry practitioners, traditional fermented food products are a viable option for probiotic delivery because they are culturally acceptable. For regulatory practitioners, scientific evidence exists to support health claims for traditionally fermented food products.

The limitations of this review are its variability in terms of quantitative methodologies used in studies, a lack of validation in clinical settings for in vitro results, and a lack of representation from certain geographical regions. The research directions to be taken in this field are to carry out human intervention studies to validate health benefits, strain-level genomic studies to reveal probiotic mechanisms of action, developing a process for probiotic fermentation to deliver probiotics in a controlled manner, and to investigate synbiotic combinations with traditional prebiotic fibers.

As the trend in gut health and functional food continues to increase in terms of interest, so will the scientific investigation of traditional fermented food products to reveal new probiotics with unique characteristics derived from centuries of food culture—offering scientific understanding and practical applications for human health.

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