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ORIGINAL ARTICLE

Probiotic Properties and Bile Salt Hydrolase Activity of Some Isolated Lactic Acid Bacteria

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ABSTRACT

Background: Lactic acid bacteria (LAB) have been studied for their possible probiotic value, particularly in enhancing intestinal health and prophylaxis of gut infections. Their survival during a stressful gastrointestinal passage and valuable relationship with a host hinge on specific properties, such as tolerance to acid and bile, enzymatic action, and antimicrobial capability. This study assessed the probiotic traits and BSH activity of probiotic bacteria strains isolated from diverse natural sources.

Methods: one hundred and four LAB isolates were derived from yogurt, curd, pickles, and infants' guts. Each isolate underwent testing for acid and bile salt tolerance, BSH activity, NaCl resistance, antimicrobial activity, auto-aggregation, and antibiotic susceptibility, along with cell surface hydrophobicity. Identification and characterization were done using standard microbiology methods. Statistical tests for significance were conducted using the chi-square test.

Results: Most isolates demonstrated strong acid (85.6%) and bile tolerance (73.1%), suggesting their ability to survive in the gastrointestinal environment. BSH activity was observed in 59.6% of the strains, while 68.3% tolerated high NaCl concentrations. Antimicrobial activity and antibiotic sensitivity were recorded in 77.9% and 83.7% of the isolates. Additionally, more than half of the isolates showed high auto-aggregation (60.6%) and hydrophobicity (55.8%), supporting their potential for intestinal adhesion.

Conclusion: Many LAB strains exhibited such probiotic characteristics as resistance to gastrointestinal conditions, pathogen suppression, and benign antibacterial properties. These results emphasize the effectiveness of LAB sourced from local regions in developing probiotic products. Additional studies utilizing living organisms are needed to confirm the claimed benefits to health.

Keywords: Lactic acid bacteria, probiotics, bile salt hydrolase, acid tolerance, antimicrobial activity, auto-aggregation, hydrophobicity, antibiotic susceptibility

INTRODUCTION

Lactic acid bacteria (LAB) are non-pathogenic and gram-positive microorganisms, and are friendly; they are well known for their use in food preparation and fermentation. Their response towards fermented dairy products, vegetables, and the intestines of humans is of special interest as it relates to their assumed biological possibilities. As per guidelines, probiotics are supposed to be live organisms that provide power and/or health attributes when taken in ample quantities. To fulfill this role, these microorganisms must survive the harsh conditions of the digestive tract, particularly low gastric pH and bile salts. They must adhere to the intestinal epithelium to exert their beneficial effects[1, 2].

The growing interest in probiotics is driven by their well-documented roles in enhancing gut health, improving immune function, and reducing the risk of certain infections. Among the various probiotic traits, tolerating acid and bile is fundamental for gastrointestinal survival. Another key attribute is bile salt hydrolase (BSH) activity, which may influence host lipid metabolism by deconjugating bile acids, potentially contributing to reducing serum cholesterol levels[3, 4].

In addition to gastrointestinal persistence, probiotics should ideally exhibit antimicrobial activity against pathogenic bacteria, demonstrate a safe antibiotic resistance profile, and possess adhesive properties that facilitate colonization of the intestinal mucosa. Such properties are commonly strain-specific and require complete evaluation before a bacterium can be considered a prospective probiotic[5, 6].

Although many commercial probiotic products are available, there is growing interest in identifying novel strains from indigenous or naturally fermented sources, particularly from traditional foods and the human microbiota. Such sources may harbor LAB strains that are functionally potent and well-adapted to regional dietary and environmental conditions[7, 8].

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This study aimed to isolate and characterize LAB from various natural sources and assess their probiotic properties, focusing on acid and bile salt tolerance, bile salt hydrolase activity, antimicrobial potential, aggregation behavior, and safety regarding antibiotic susceptibility. By evaluating these functional characteristics, this study seeks to identify promising LAB strains that could contribute to the development of effective probiotic formulations.

METHODOLOGY

This experimental study was conducted over six months, from March 2023 to August 2023, at the Microbiology Department of Hayatabad Medical Complex, Peshawar. No formal ethical approval was required as the study involved microbial samples from non-invasive sources such as food and feces. However, all protocols were conducted by institutional biosafety standards.

One hundred four samples were collected from various natural and fermented sources, including yogurt, curd, pickle brine, and infant feces. All samples were transported under chilled conditions and processed within 24 hours. Serial dilutions were prepared using sterile saline, and aliquots were plated on de Man, Rogosa, and Sharpe (MRS) agar, which supports the growth of lactic acid bacteria. The plates were incubated at 37°C for 48 hours under anaerobic conditions. Colonies with distinct morphology were selected and sub-cultured for purification.

Purified isolates were subjected to Gram staining and catalase testing. Only Gram-positive, catalase-negative isolates were selected for further analysis, consistent with the basic identification criteria of LAB. Cell morphology (rod or cocci) was recorded through microscopy. Biochemical profiling and genus-level identification were performed using standard carbohydrate fermentation tests.

All selected LAB isolates underwent a series of in vitro tests to assess their probiotic potential:

- Acid Tolerance: Isolates were exposed to MRS broth adjusted to pH 2.0 and incubated for 2 hours. Viable colony counts were recorded post-incubation to assess survival under gastric conditions.
- Bile Salt Tolerance: Growth of isolates was evaluated in MRS broth supplemented with 0.3% bile salts. Viability was determined after 4 hours to simulate small intestinal conditions.
- Bile Salt Hydrolase (BSH) Activity: BSH activity was tested by streaking isolates on MRS agar containing bile salts and calcium chloride. After 72 hours of anaerobic incubation, the opaque halos around colonies indicated positive BSH activity.
- Salt Tolerance: NaCl resistance was determined by culturing the isolates in MRS broth with increasing salt concentrations (2%, 4%, and 6.5%). Growth was assessed visually and by measuring optical density at 600 nm.
- Antimicrobial Activity: The agar well diffusion method was used to assess the ability of LAB isolates to inhibit common pathogenic bacteria, including E. coli, Salmonella typhi, and Staphylococcus aureus. Zones of inhibition were measured in millimeters.
- Antibiotic Susceptibility: Disc diffusion testing was performed using commercially available antibiotic discs on Mueller-Hinton agar. The susceptibility or resistance of each isolate was interpreted according to CLSI guidelines.
- Auto-aggregation: Cultures were grown in MRS broth and allowed to settle. The degree of auto-aggregation was calculated as a percentage decrease in optical density of the upper suspension layer over 4 hours.
- Hydrophobicity: The isolates' ability to adhere to hydrocarbons (xylene) was assessed. A decrease in the aqueous phase's absorbance indicated higher cell surface hydrophobicity, which correlates with adhesion potential.

All experiments were performed in triplicate to ensure reliability. The results were recorded as mean percentages or frequencies and analyzed using SPSS version 25. Chi-square tests were applied to determine the significance of observed differences among isolates, with a p-value of less than 0.05 considered statistically significant.

RESULT

Most lactic acid bacteria isolates in this study were sourced from yogurt and curd, accounting for nearly two-thirds of the total samples. This reflects the well-established presence of probiotic strains in traditional dairy products. A smaller proportion came from pickle brine and infant gut, showing the diversity of environments where LAB can thrive. All isolates were confirmed to be Gram-positive and catalase-negative—hallmark features of LAB—while rod-shaped morphology predominated. Lactobacillus emerged as the most frequently identified group, reinforcing its reputation as a robust probiotic candidate. These initial findings provide a strong baseline for evaluating their functional properties.

Table 1: Source and Identification Characteristics of LAB Isolates (n = 104)

Variable	Categories	Frequency (%)
Source of Isolation	Yogurt	38 (36.5%)
	Curd	30 (28.8%)
	Pickle Brine	20 (19.2%)
	Infant Gut	16 (15.3%)
Gram Reaction	Gram-Positive	104 (100%)
Cell Morphology	Rods	88 (84.6%)
	Cocci	16 (15.4%)
Catalase Test	Negative	104 (100%)
Genus Identified	Lactobacillus spp.	65 (62.5%)
	Leuconostoc spp.	23 (22.1%)
	Pediococcus spp.	16 (15.4%)

Survival under acidic and bile conditions is critical for a microorganism to be considered probiotic. In this study, a substantial proportion of isolates (85.6%) survived at pH 2, simulating the stomach's highly acidic environment. Similarly, 73.1% tolerated the presence of bile salts, mimicking conditions in the small intestine. The statistical significance (p-values < 0.05) suggests that these traits are not due to chance and are likely inherent characteristics of the selected LAB strains. These results support the potential of these isolates to reach the intestinal tract alive, which is essential for probiotic efficacy.

Table 2: Acid and Bile Salt Tolerance of LAB Isolates

Parameter	Tolerant Isolates (n, %)	Non-tolerant Isolates (n, %)	p-value
Acid Tolerance (pH 2)	89 (85.6%)	15 (14.4%)	0.032
Bile Salt Tolerance	76 (73.1%)	28 (26.9%)	0.047

Approximately 60% of the tested LAB isolates showed bile salt hydrolase (BSH) activity. This enzymatic function is beneficial because it helps deconjugate bile acids, possibly reducing cholesterol and enhancing intestinal health. Additionally, 68.3% of the strains survived in a 6.5% NaCl environment, demonstrating their resilience to salt stress. This trait is crucial for strains intended for incorporation into fermented foods, where salt is commonly used. The statistically significant outcomes further affirm that many strains possess functional and industrially relevant characteristics.

Table 3: Bile Salt Hydrolase (BSH) Activity and NaCl Tolerance

Parameter	Positive Isolates (%)	Negative Isolates (%)	p-value
Bile Salt Hydrolase Activity	62 (59.6%)	42 (40.4%)	0.041
NaCl Tolerance (6.5%)	71 (68.3%)	33 (31.7%)	0.028

Table 4: Antimicrobial and Antibiotic Susceptibility Profile

Parameter	Positive Response (%)	Negative Response (%)	p-value
Antimicrobial Activity	81 (77.9%)	23 (22.1%)	0.019
Antibiotic Sensitivity	87 (83.7%)	17 (16.3%)	0.022

Table 5: Aggregation and Hydrophobicity Properties

Parameter	High Activity (%)	Low/No Activity (%)	p-value
Auto-Aggregation (>50%)	63 (60.6%)	41 (39.4%)	0.036
Cell Surface Hydrophobicity	58 (55.8%)	46 (44.2%)	0.049

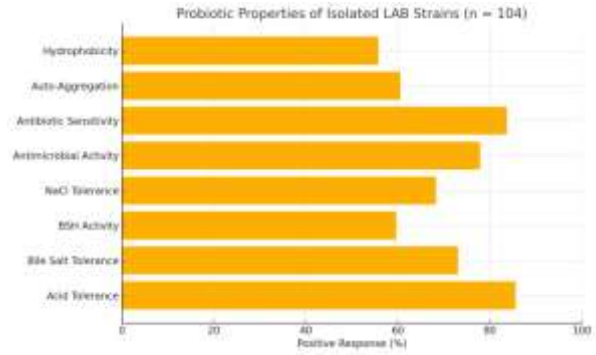

Figure 1: The graph shows that most LAB isolates performed well in key probiotic tests. The most positive rates were found in acid tolerance and antibiotic sensitivity, demonstrating elevated survival ability and safety. High antimicrobial activity was also noted, suggesting that these strains can inhibit opportunistic pathogens. Tolerance to bile salt and NaCl was moderate, indicating fair resilience under stress. More than 50 percent of the strains possessed bile salt hydrolase activity, aggregation, and hydrophobicity, supporting their probiotic potential via adhesion and survival in the intestines. These were consistent and optimal functional traits for the strains.

Most LAB isolates exhibited potent antimicrobial activity, with 77.9% effectively inhibiting the growth of pathogenic organisms. This feature is fundamental for preserving gut health through the antagonism of harmful bacteria. Additionally, 83.7% of isolates were sensitive to standard antibiotics, which is a desirable trait for probiotics to ensure they do not contribute to antibiotic resistance. The statistical significance of both findings confirms the relevance of these isolates not only in food safety but also in clinical applications. Their dual ability to combat pathogens while remaining susceptible to antibiotics strengthens their case as safe and effective probiotics.

A key component of probiotic functionality is the ability of bacteria to adhere to intestinal cells, which enhances colonization and interaction with the host. In this study, 60.6% of isolates demonstrated high auto-aggregation, indicating good cell-to-cell adhesion, while 55.8% displayed significant cell surface hydrophobicity. These properties are directly linked to the ability to attach to epithelial surfaces in the gut. The statistically significant results provide additional support for the probiotic potential of these strains, suggesting they are resilient and functional and capable of establishing themselves in the host's gastrointestinal environment.

DISCUSSION

This study investigated the probiotic properties and bile salt hydrolase activity of 104 lactic acid bacteria (LAB) isolates from natural sources. The findings support that many of these isolates have requisite attributes to be utilized as probiotics[9-11].

This study's most notable finding was the remarkable acid and bile salt tolerance in most LAB strains. These traits are critical for survival since they allow the bacteria to withstand the acidic conditions of the stomach as well as the bile salt-rich environment of the small intestine. Similar results were recorded with studies claiming that more than 70% of the LAB strains obtained from traditional Indian fermented foods demonstrated high resistance to acid and bile[12-14]. This congruence strengthens the claim regarding the naturally obtained LAB enduring gastrointestinal challenges.

The expression of bile salt hydrolase (BSH) activity in almost 60% of isolates is essential considering its possible function in cholesterol lowering and bile acid detoxification. Similar results were found in studies that highlighted the probiotic importance of BSH-positive strains in host lipid metabolism modulation[11-13]. Additionally, the capacity of LAB to endure high salt concentrations (up to 6.5%) supports the hypothesis of their industrial applicability, particularly about salted or fermented food products.

The visceral antimicrobial activity from microbial gut isolates indicates their ability to inhibit some common gut pathogens. This antagonism is usually linked with the secretion of organic acids, hydrogen peroxide, and bacteriocins. Studies have likewise pointed out that various Lactobacillus spp. from dairy sources strongly inhibited pathogens[15-17].

As previously stated, auto-aggregation and hydrophobicity tests were conducted to evaluate adhesion to intestinal mucosa, a highly important factor for probiotic evaluation. More than half of the isolates showed significant amounts of auto-aggregation and hydrophobicity, which suggests that they have good potential for colonization in the gut. Other studies indicate that these traits assist probiotics that need to interact with the host in optimizing interaction with epithelial cells and thus enhancing their competitiveness and effectiveness[18-20].

In conclusion, the sensitivity profiles of most isolates correspond with their safety profile. Unlike resistant strains, which may contribute to horizontal gene transfer, the sensitivity of these isolates to antibiotics mitigates the dangers of using foods derived from these isolates or employing them in clinical settings.

CONCLUSION

The present study illustrates that numerous Lactic Acid Bacteria (LAB) isolates derived from natural sources possess probiotic traits such as tolerance to acid and bile, antimicrobial properties, bile salt hydrolase activity, and good adhesion ability. Their sensitivity to antagonistic drugs emphasizes their safety and lack of harm to human beings. These results warrant using the isolates to develop functional foods or dietary supplements based on probiotics. Studies in animal models alongside genetic characterization of the strains at the level of alleles are necessary to confirm these findings and ascertain the best candidates for commercial exploitation.

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