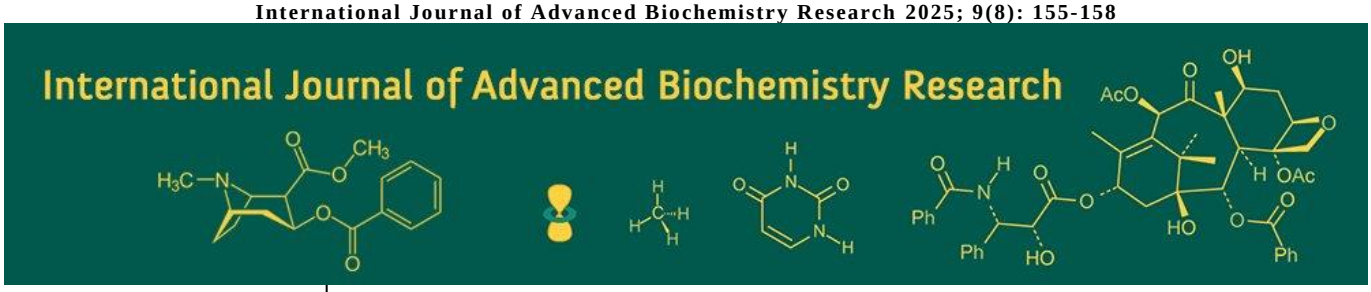

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Isolation and characterization of lactic acid bacteria from homemade curd samples of Jammu district

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Abstract
Homemade curd is a naturally fermented dairy product that serves as an important habitat for diverse lactic acid bacteria (LAB), many of which are valuable for probiotic and functional food applications. In the present investigation, homemade curd samples were collected from different localities of Jammu district to explore indigenous LAB diversity. A total of 10 samples were processed under aseptic conditions, and serial dilutions were plated on de Man, Rogosa, and Sharpe (MRS) agar for isolation. From these samples, 20 morphologically distinct bacterial colonies were recovered. All isolates were initially characterized by Gram staining and catalase tests, which confirmed that they were Gram-positive, catalase-negative, and non-spore-forming, typical of LAB. Detailed morphological and biochemical analyses were performed, including sugar fermentation patterns, esculin hydrolysis, and citrate utilization. Out of the 20 isolates, seven strains consistently displayed characteristics typical of LAB, with uniform rod-shaped morphology and stable biochemical responses. These seven promising isolates were further subjected to species-level identification using the ABIS bacterial identification system, which classified all of them as members of the genus *Lactiplantibacillus*, a group widely recognized for its role in fermentation and probiotic functionality. The findings highlight that homemade curd from Jammu is a rich source of indigenous LAB, and the *Lactiplantibacillus* isolates obtained in this study may serve as potential candidates for starter culture development and probiotic applications after further safety and functional evaluations.

Keywords: Lactic acid bacteria, homemade curd, Jammu district, *Lactiplantibacillus*, isolation, ABIS identification

Introduction
Lactic acid bacteria (LAB) are a diverse group of Gram-positive, catalase-negative, non-spore-forming microorganisms that play a pivotal role in the fermentation of dairy and other food products. Their main metabolic activity involves converting sugars into lactic acid, which improves food safety, texture, and flavour while extending shelf life (Gänzle, 2015) [1]. In addition to their technological functions, several LAB species are recognized as probiotics, capable of modulating the gut microbiota, enhancing host immunity, and providing protective effects against pathogenic microorganisms (Saad *et al.*, 2013) [1].
Homemade curd, commonly known as “dahi” in India, is prepared through natural back-slopping using a portion of previously fermented curd as the inoculum. Its microbial composition is highly diverse and largely influenced by factors such as milk source, hygiene, and regional preparation practices (Patel *et al.*, 2020) [3]. Unlike industrial yogurt, which relies on defined starter cultures, traditional curd represents a reservoir of indigenous LAB with potential technological and probiotic applications.
Screening native LAB strains from traditional dairy products is crucial because such isolates often exhibit desirable properties, including tolerance to acidic pH, bile salts, and salinity, along with the ability to produce antimicrobial compounds. These traits are essential for survival in the gastrointestinal tract and for use in functional food formulations (Ranadheera *et al.*, 2017; Kumar *et al.*, 2021) [4, 5]. Among the LAB genera, *Lactiplantibacillus* and Lacticaseibacillus are widely reported for their significance in dairy fermentation and probiotic product development (Aghdasian *et al.*, 2022) [6].
Considering the importance of local microbial biodiversity, this study was conducted to isolate and characterize LAB from homemade curd samples collected across various regions of Jammu district. Emphasis was placed on morphological and biochemical characterization,

followed by ABIS-based identification, to select strains with promising technological and probiotic potential.

2. Materials and Methods

2.1 Sample Collection and Isolation of LAB

A total of 20 homemade curd samples were collected aseptically from different localities of Jammu district to capture the regional microbial diversity. Fresh curd (approximately 50 mL) was collected in sterile containers, transported to the laboratory under refrigerated conditions (4 °C), and processed within 24 hours. For isolation, 1 g of each curd sample was homogenized in 9 mL of sterile physiological saline (0.85% NaCl) to obtain a 10^{-1} dilution. Serial dilutions were prepared up to 10^{-6}. Aliquots (100 µL) from appropriate dilutions were spread plated on de Man, Rogosa and Sharpe (MRS) agar (HiMedia®) and incubated anaerobically at 37 °C for 24-48 hours. Distinct colonies were subcultured repeatedly on MRS agar to obtain pure LAB isolates, which were preserved in MRS broth containing 20% glycerol at −20 °C for further analysis.

2.2 Characterization of LAB

2.2.1 Morphological Characterization

Colonies of LAB on MRS agar typically appeared small, smooth, creamy-white, and circular. Distinct colonies were subjected to Gram staining to confirm the presence of Gram-positive rods or cocci, which is a hallmark of LAB. Motility testing was performed using hanging drop method and confirmed that all isolates were non-motile, which is characteristic of LAB.

2.2.2 Biochemical Characterization

Biochemical profiling of the isolates was performed using HiLco™ KB020 kits (HiMedia, India), designed for the identification of bacterial strains based on their metabolic activities and carbohydrate utilization patterns. Preparation of inoculum: Pure cultures were first grown on MRS agar slants and incubated for 24-48 h at 37 °C. Inoculation and incubation: A loopful of each isolate was inoculated into the 12-substrate biochemical panels and incubated at 37 °C for 18-24 h. Result interpretation: A colour changes from red to yellow indicated acid production from carbohydrate fermentation, which was recorded as a positive reaction. The biochemical profiles were compared with reference patterns and further analysed using ABIS (Automated Bacterial Identification System) to obtain probable species-level identification.

2.5 Selection of Promising Isolates

Based on consistent LAB-typical morphology (Gram-positive rods, catalase-negative), stable and expected biochemical profiles (sugar fermentation, esculin hydrolysis), and absence of aberrant traits, seven isolates showing uniform and robust characteristics were shortlisted for further identification.

2.6 Genus-Level Identification via ABIS

The seven promising isolates were subjected to in silico identification using the ABIS (Automated Bacterial Identification System) platform. For this, biochemical profile data and morphological attributes were formatted as required and submitted to ABIS. The output identification, including probable genus and species assignments with confidence scores, was recorded. All seven isolates were identified as members of the genus *Lactiplantibacillus*.

2.7 Quality Control and Data Recording

All media were prepared according to manufacturer instructions and sterilized by autoclaving at 121 °C for 15 minutes. Positive controls (reference LAB strains where available) and negative controls (non-LAB bacteria for catalase) were included to validate test performance. Data were logged systematically, and ambiguous or inconsistent isolates were excluded from the selected seven.

3. Results

3.1 Isolation of LAB from Homemade Curd

Out of 20 homemade curd samples 10 LAB isolates were isolated on MRS agar. The colonies were generally small, circular, smooth, and creamy-white. All isolates were Gram-positive, catalase-negative, and non-spore-forming, confirming their preliminary identification as LAB. Motility tests confirmed that all isolates were non-motile, consistent with LAB characteristics.

Table 1: Morphological characteristics of LAB isolates from homemade curd

Isolate Code	Colony Color	Shape	Elevation	Margin	Gram Stain	Catalase	Motility
L1	Creamy-white	Circular	Convex	Smooth	+	-	-
L2	Creamy-white	Circular	Convex	Smooth	+	-	-
L3	Creamy-white	Circular	Convex	Smooth	+	-	-
L4	Creamy-white	Circular	Convex	Smooth	+	-	-
L5	Creamy-white	Circular	Convex	Smooth	+	-	-
L6	Creamy-white	Circular	Convex	Smooth	+	-	-
L7	Creamy-white	Circular	Convex	Smooth	+	-	-
L8	Creamy-white	Circular	Convex	Smooth	+	-	-
L9	Creamy-white	Circular	Convex	Smooth	+	-	-
L10	Creamy-white	Circular	Convex	Smooth	+	-	-

Biochemical Characterization

As shown in table 2 using HiMedia® biochemical test kits revealed uniform metabolic behaviour among the isolates. All ten isolates (L1-L10) were identified as *Lactiplantibacillus* spp. based on their biochemical characteristics. Most showed positive results for esculin hydrolysis and were catalase-negative, which is typical for this genus. They demonstrated the ability to ferment a wide range of sugars including sucrose, glucose, fructose, maltose, galactose, and mannose. Variations were noted in arabinose and melibiose fermentation, with isolate L5 showing the most limited metabolic activity. Isolates L7 and L8 were unique in their ability to ferment raffinose. Overall, the fermentation patterns and enzyme activities support the identification of these isolates as members of the *Lactiplantibacillus* genus supporting their potential classification as probiotic lactic acid bacteria.

Table 2: Biochemical profiling of the isolated LAB strains

S. No.	Isolates	Substrate used													Identification on the basis of ABIS online software
		Esculin hydrolysis	Catalase	Xylose	Cellobiose	Arabinose	Maltose	Galactose	Mannose	Mellibiose	Raffinose	Sucrose	Sucrose	Trehalose	
1	L1	+	-	+	+	+	+	+	+	-	-	+	+	+	*Lactiplantibacillus* spp.
2	L2	+	-	+	+	-	+	+	+	-	-	+	+	+	*Lactiplantibacillus* spp.
3	L3	+	-	-	+	+	+	+	+	-	-	+	+	+	*Lactiplantibacillus* spp.
4	L4	+	-	+	+	+	+	+	+	+	-	+	+	+	*Lactiplantibacillus* spp.
5	L5	-	-	-	+	-	+	+	+	-	-	+	+	-	*Lactiplantibacillus* spp.
6	L6	+	-	+	+	+	+	+	+	±	-	+	+	+	*Lactiplantibacillus* spp.
7	L7	+	-	+	+	+	+	+	+	-	+	+	+	+	*Lactiplantibacillus* spp.
8	L8	+	-	+	+	+	+	+	+	-	+	+	+	+	*Lactiplantibacillus* spp.
9	L9	+	-	+	+	+	+	+	+	-	-	+	±	+	*Not identified.*
10	L10	-	-	+	-	-	+	+	+	-	-	+	+	+	*Not identified*

+ = Positive (substrate used or enzyme present)
- = Negative (no use or enzyme absent)
± = Variable/weak reaction

3.3 Selection of Promising LAB Isolates

Based on consistent morphology (Gram-positive rods, catalase-negative, non-motile), stable biochemical traits (acid fermentation of sugars, esculin hydrolysis), and absence of aberrant reactions, seven isolates (L1-L7) were selected as promising LAB candidates for further identification.

3.4 Genus-Level Identification using ABIS

The biochemical and morphological data of the seven selected isolates were entered into the ABIS (Automated Bacterial Identification System). The system consistently assigned all seven isolates to the genus *Lactiplantibacillus* with confidence scores ranging between 90-97%.

Table 3: ABIS-based genus-level identification of promising LAB isolates

Isolate Code	ABIS Identification	Confidence (%)
L1	*Lactiplantibacillus*	97
L2	*Lactiplantibacillus*	95
L3	*Lactiplantibacillus*	96
L4	*Lactiplantibacillus*	93
L5	*Lactiplantibacillus*	92
L6	*Lactiplantibacillus*	91
L7	*Lactiplantibacillus*	90

3.5 Quality Control

All culture media were sterilized under standard conditions and quality checks were maintained. Positive controls (reference LAB strains) confirmed expected Gram and catalase reactions, while negative controls (non-LAB bacteria) validated the specificity of tests

4. Discussion

The current study investigated the microbial diversity of homemade curd from various regions of Jammu district with the aim of isolating and characterizing lactic acid bacteria (LAB). Out of 10 isolates obtained, seven were identified as promising strains based on their morphological and biochemical traits and further confirmed through ABIS identification.

All isolates were Gram-positive, catalase-negative, and non-spore-forming, which are hallmark characteristics of LAB widely reported in naturally fermented dairy products (Patel *et al.*, 2020) [3]. The creamy-white, convex colonies with smooth edges observed on MRS agar correspond well with the morphological features of *Lactiplantibacillus* and *Lacticaseibacillus* species frequently associated with artisanal curd (Ranadheera *et al.*, 2017).

Biochemical analysis demonstrated universal glucose and lactose fermentation, while the ability to utilize sucrose, maltose, and mannitol varied across isolates. This pattern is typical for LAB and reflects species-specific carbohydrate metabolism, which is often used for preliminary differentiation (Kumar *et al.*, 2021) [5]. Esculin hydrolysis was positive, whereas citrate utilization was negative for all isolates, consistent with their adaptation to the milk environment.

The seven selected isolates were identified as *Lactiplantibacillus* Thes genus are frequently reported as probiotic candidates due to their tolerance to gastrointestinal stress, production of exopolysaccharides, and antimicrobial effects against pathogens (Aghdasian *et al.*, 2022) [6]. The dominance of *Lactiplantibacillus* spp. in homemade and traditionally fermented curd has also been observed in similar studies, highlighting the potential of such products as natural reservoirs of functionally relevant LAB (Saad *et al.*, 2013; Zannini *et al.*, 2016) [1, 7].

Overall, the study confirms that homemade curd from Jammu harbors indigenous LAB strains with notable probiotic potential. These isolates may serve as valuable candidates for starter culture development and functional dairy products, although further evaluation of safety and *in vitro* probiotic functionality is necessary to validate their commercial application.

5. Conclusion

The present study demonstrated that homemade curd from different regions of Jammu district serves as a natural reservoir of lactic acid bacteria (LAB) with desirable characteristics. From 20 curd samples, a total of 20 LAB isolates were obtained, all of which were Gram-positive, catalase-negative, and non-motile, consistent with typical LAB traits. Biochemical profiling revealed consistent glucose and lactose fermentation, variable utilization of other sugars, and universal esculin hydrolysis with negative citrate utilization, confirming their adaptation to the dairy environment. Based on morphological and biochemical stability, seven isolates (L1-L7) were shortlisted as promising LAB candidates. ABIS identification confirmed all seven isolates as members of the genus *Lactiplantibacillus*, a group well recognized for its role in dairy fermentations and probiotic applications.

The findings highlight that indigenous LAB from traditional curd are functionally relevant and hold potential as starter cultures or probiotic strains. Further studies, including molecular identification, probiotic functionality assays (acid/bile tolerance, antimicrobial activity), and safety

evaluation, are necessary to establish their suitability for functional food development.

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