Indonesian Journal of Medical Laboratory Science and Technology
Open Access
p-ISSN 2684-6748
e-ISSN 2656-9825

RESEARCH ARTICLE

Quality assessment and biochemical characterization of probiotic *Lactobacillus* species isolated from yogurts fermented either by *Tamarindus indica* or *Capsicum annuum*

Sowmiya Thiyagarajan [1], Emmanuel Bhaskar [2], Veeraraghavan Gayathri [3], Parthasarathy Mohanalakshmi [4], Santhi Silambanan [4]

[1]Department of Biochemistry, Sri Ramachandra Institute of Higher Education and Research, Chennai, India
[2]Department of General Medicine, Sri Ramachandra Institute of Higher Education and Research, Chennai, India
[3]CEFTE, Sri Ramachandra Institute of Higher Education and Research, Chennai, India
[4]Department of Biochemistry, Sri Muthukumaran Medical College and Research Institute, Chennai, India

Correspondence:
Santhi Silambanan
Department of Biochemistry,
Sri Ramachandra Institute of Higher Education and Research (SRIHER) (DU)
Chennai, Tamil Nadu, India

Email: santhisilambanan@gmail.com

Article history:
Received: 2024-07-23
Revised: 2024-12-27
Accepted: 2025-01-16
Available online: 2025-04-27

Keywords:
16S rRNA
Microbiota
Probiotic yogurt
Proximate principles

https://doi.org/10.33086/ijmlst.v7i1.6280

Yogurt is used to treat various digestive ailments. However, there are limited studies of the biochemical and microbiological properties of yogurt. This study aimed to assess the quality and biochemical characteristics of probiotic *Lactobacillus* species isolated from yogurts fermented with *Tamarindus indica* or *Capsicum annuum*. Three yogurt samples were used for analysis; two were homemade, using either *Tamarindus indica* L. or *Capsicum annuum* L. to initiate fermentation. Third was a commercial yogurt obtained from the grocery market. A proximate analysis was carried out. To identify microbiological strains, yogurt samples were cultured on nutrient- and De Man-Rogosa-Sharpe agars. Biochemical characteristics were examined, and probiotic species were identified through 16S rRNA analysis. The results showed that traditional yogurts had lower energy content due to reduced carbohydrate and fat levels while exhibiting higher protein content. *Escherichia coli* and *coliforms* were detected in the commercial yogurt, whereas traditional yogurts were free from these harmful bacteria, highlighting their potential safety. The biochemical characteristics of all yogurt samples were largely similar. *C. annuum* L. demonstrated a positive oxidase test, indicating the presence of an electron transport chain in the Lactobacillus species found in the yogurt fermented with *C. annuum* L. Microbial analysis revealed that *L. acidophilus* was predominant in traditional yogurt, while commercial yogurt contained *L. plantarum.* These findings suggest that homemade yogurts, with their higher protein content, lower fat and carbohydrate levels, and absence of harmful bacteria, offer a safer and potentially more beneficial probiotic alternative to commercial yogurt. The specific Lactobacillus strains present in traditional yogurts may contribute to their antimicrobial properties, supporting their probiotic potential.

1. INTRODUCTION

Yogurt, a key component of Asian culinary traditions for centuries, particularly in regions like the Central Asia, the Middle East, and parts of South Asia (1) has a history that can be traced back thousands of years. Its origins are believed to date to ancient times, when nomadic cultures played a crucial role in discovery of the natural fermentation process of milk, leading to the creation of yogurt. Yogurt is commonly made from the milk of cow, sheep, buffalo, goat, camel, and other domestic animals (2). Initially, the use of *Lactobacillus* species in fermenting milk was primarily for preservation purposes, thus extending the shelf life of dairy products in the absence of modern refrigeration methods. This process was observed and refined over generations, leading to the deliberate creation of yogurt as a distinct food item (3).

Citation: Thiyagarajan S, Bhaskar E, Gayathri V, Mohanalakshmi P, Silambanan S. Quality assessment of probiotic *Lactobacillus* species isolated from yogurts fermented either by *Tamarindus indica* or *Capsicum annuum*. Indones J Med Lab Sci Technol. 2025;7(1):60–69. https://doi.org/10.33086/ijmlst.v7i1.6280

In recent years, understanding *Lactobacillus* and its role in promoting health benefits has evolved significantly. While historically, it was used for fermentation and preservation. Modern research has identified specific strains of *Lactobacillus* as probiotics. Probiotics are living microorganisms that offer health benefits when consumed in adequate amounts and should contain at least 10^6 CFU/mL viable microbial load, according to the International Scientific Association for Probiotics and Prebiotics (ISAPP) (4). This transition from merely a fermentation agent to a recognized probiotic illustrates a profound shift in our understanding of how certain microorganisms positively impact human health. In Turkey, there is a unique variety of yogurt known as cone yogurt, which is distinguished by its fermentation process that involves solely the addition of pine cones to milk, without using of any cultures (5). This particular yogurt has a longstanding tradition and has been crafted for numerous years, typically by a select group of producers situated in specific mountain villages within Turkey's Kutahya and Eskisehir regions (6). For generations, our ancestors crafted homemade yogurt made from *Tamarindus indica* L. (tamarind) and *Capsicum annuum* L. (chili), rich in probiotics, unaware of its remarkable health benefits (7,8). Probiotic yogurts which can either by easily prepared at home without expensive starter cultures or commercially procured, and these traditional practices harbored hidden health advantages.

The study's novelty was that altered gut microbiota has been found to have significant pathogenesis in various disease states such as diabetes mellitus, major depressive disorder, obesity, nonalcoholic fatty liver disease, and insulin resistance. Balancing microbiota with prebiotics and probiotics is essential in handling treatment-resistant cases (9). This research aimed to unveil the benefits by isolating and identifying *Lactobacillus* strains in homemade traditional probiotic yogurt, providing a foundation for future investigations on their health effects.

2. MATERIALS AND METHODS

In this study, three yogurt samples were used for analysis. Homemade samples were prepared daily at home. Fermentation was initiated using either *T. indica* L. or *C. annuum* L. to prepare homemade yogurt samples. Commercial yogurt samples were freshly purchased daily from the same public grocery shop. Proximate analysis was conducted on all yogurt samples, including the energy value, carbohydrate, total fat, protein, moisture, ash, fiber, total solids, and titratable acidity. The microbiological strains were examined by culturing the yogurt samples in nutrient agar and De Man-Rogosa-Sharpe agar (HiMedia Laboratories Private Ltd). All three samples underwent biochemical characterization of the cultures obtained. Probiotic species present in the yogurt samples were identified through 16S rRNA analysis.

2.1. Sampling and Preparation of Traditional Probiotic Yogurt Using *Tamarindus indica* L. and *Capsicum annuum* L.

In the southern states of India, homemade traditional yogurts are prepared from cows' milk using *T. indica* L. and *C. annuum* L. to initiate fermentation (10,11). It is an easy process used since ancient times and passed through generations. The process involves heat treatment, cooling to an incubation temperature, and then subjected to fermentation. Milk was boiled for 15 minutes to eliminate to the pathogens as well as to denature the whey protein. The milk was removed from the burner and left at room temperature (37°C) for the milk to reach a temperature of 40-45°C. Then, *T. indica* L. (30 grams) or *C. annuum* L. (2 numbers) was introduced into 500 mL milk, stirred gently, and left to ferment for 10-12 hours. After fermentation, a portion of the yogurt was utilized to form further yogurt. In order to investigate whether the presence of *Lactobacillus* species is attributable to the microorganisms present in *T. indica* L. or *C. annuum* L., this study used this back-slopping technique for up to 10 preparations (12). The yogurts traditionally produced by the back-slopping method are shown in Figure 1. There was a remarkable increase in colony-forming units (CFU) with subsequent fermentations. Also, there were enhancements in texture and thickness with each successive generation. The traditional yogurt prepared with fermentation of *T. indica* or *C. annuum* is shown in Figure 2.

2.2. Proximate Analysis of Yogurt

The energy value, carbohydrate, total fat, protein, moisture, ash, fiber, total solids, and total titratable acidity (TTA) were determined using Association of Official Analytical Chemists (AOAC) International guidelines (13,14). The energy content was determined using a bomb calorimeter by measuring the heat released during combustion. The carbohydrate content was indirectly calculated by subtracting the sum of protein, moisture, ash, and fat from 100%. The extraction of fat from yogurt involved the use of Soxhlet extraction with petroleum ether. The percentage of total fat was calculated based on the weight of extracted fat with the initial weight of the yogurt sample. The AOAC Kjeldahl method was employed for protein analysis, involving yogurt sample digestion with concentrated sulfuric acid (H_2SO_4) to release ammonia (NH_3), subsequently titrated using standardized HCl. Protein percentage was calculated by determining the nitrogen content obtained from the titration. Moisture content was determined by oven-drying five-gram yogurt sample at 105°C until a stable weight had reached. The loss in weight represented the moisture content. Ash content was obtained by incinerating yogurt samples in a muffle furnace at 550°C until complete combustion of organic matter occurred yielding inorganic ash content. Fiber content was determined by treating the sample with H_2SO_4 and NaOH to eliminate non-fibrous elements. The resulting residues were filtered, dried, and weighed. Total solids were determined by drying 10 grams of yogurt sample

until a constant weight was achieved. The TTA is a measure of the amount of lactic acid in the yogurt, and was expressed as a percentage. TTA was determined by titrating 10 grams of yogurt sample with standardized NaOH and phenolphthalein indicator. The amount of NaOH consumed indicated the acidity. The samples were also tested for their pH and sensory characteristics (15,16).

2.3. Safety and Quality Profile of Traditional Yogurts and Commercial Yogurt

The quality of yogurt is based on its palatability and sensory properties, such as texture, color, and appearance. Therefore, establishing tolerance limits for these attributes is essential in yogurt production to ensure consistent product quality. The typical sensory characteristics of yogurt include creamy or white color, soft and smooth semi-solid structure, spreadable or spoonable consistency, and slightly acidic, sour taste. These properties are influenced by protein content, fermentation processes, and texture (17).

As much as 0.1 mL of each yogurt sample was inoculated to the agar surface using an L-rod. The plates were incubated for 24-48 hours at 37°C. Isolated colonies were sub-cultured on freshly prepared MRS agar plates to obtain a pure *Lactobacillus* culture. Colony characteristics, colony count, Gram staining, and microscopic morphological features of the isolates were analyzed. Both traditional yogurt samples and commercial yogurt sample were examined for the presence of common pathogenic microorganisms. Selective media, including membrane Filter Fecal Coliform Agar (mFC) and MacConkey agar (MAC), were used to identify *Escherichia coli* and *coliforms*, while Sabouraud Dextrose Agar (SDA) was employed to identify fungi and yeasts. These media were specifically formulated to promote the growth of *E. coli*, coliform bacteria, and fungi (18,19).

2.4. Biochemical Characteristics of Microbial Isolates

The microbial isolates from the three yogurt samples were tested for biochemical characteristics based on the method by Rahman et al (20).

2.4.1. Indole test

The tryptone broth (10 grams of Tryptone dissolved in 1000 mL of distilled water) was inoculated with the test isolates. This mixture was incubated at 37°C for 24-48 hours. After incubation, approximately 0.2-0.3 mL of Kovac's reagent (5 grams para-dimethyl amino Benzaldehyde, 75 mL butyl alcohol, and 25 mL concentrated HCl), was added. The mixture was thoroughly mixed and left to stand for 30 minutes. The formation of a brown ring on the broth's surface indicated the presence of indole production (21).

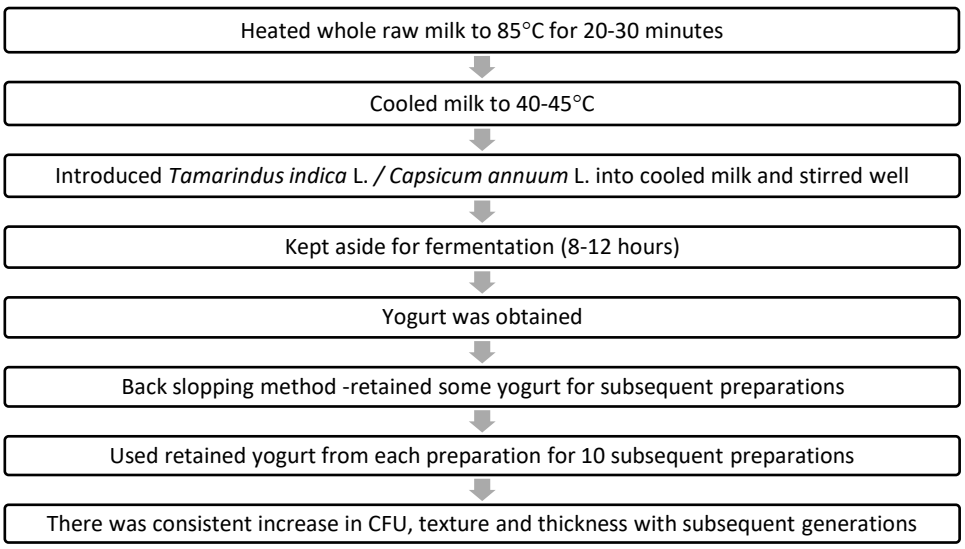

Figure 1. Preparation of traditional probiotic yogurt using *Tamarindus indica* L. and *Capsicum annuum* L.

Figure 2. Traditional probiotic yogurt prepared with fermentation of *Tamarindus indica* L. or *Capsicum annuum* L.

2.4.2. Methyl red test

The Methyl red-Voges Proskauer (MR-VP) broth (7 g/L buffered peptone, 5 g/L dextrose, 5 g/L K_2HPO_4, 5 g/L NaCl, and adjusted to a pH of 7.2), was inoculated with the culture and incubated at 37°C for 24-48 hours. The appearance of red color with the addition of methyl red reagent was considered positive. The color change denoted the presence of microorganisms participating in the mixed-acid fermentation pathway (21).

2.4.3. Voges-Proskauer test

The MR-VP medium inoculated with the culture was incubated at 37°C for 24-48 hours. After incubation, 3 mL of Barrit's reagent A (5% alpha naphthol in 95 mL of absolute ethanol) and 1 mL of Barrit's reagent B (40 g/L KOH, 3g/L) were added. The tubes were agitated and then left undisturbed for 15 minutes. The appearance of red color indicated a positive test (21).

2.4.4. Urease test

The urease agar medium (1 g/L dextrose, 1.5 g/L peptic digest of animal tissue, 5 g/L NaCl, 2 g/L KH_2PO_4, 0.012g/L phenol red, 15 g/L agar, with a final pH of 6.8±0.2 at 25°C), was inoculated with the culture and incubated for 24 hours at 37°C. During this incubation period, the breakdown of urea led to the production of NH_3, causing the medium to become alkaline and exhibit a pink-red coloration (21).

2.4.5. Triple sugar iron test (TSI)

The TSI agar medium (3 g/L beef extract, 20 g/L peptone, 3 g/L yeast extract, 10 g/L lactose, 10 g/L sucrose, 1 g/L dextrose monohydrate, 0.2 g/L $FeSO_4$, 5 g/L NaCl, 0.3 g/L $Na_2S_2O_3$, 0.024 g/L phenol red, 12 g/L agar, adjusted to a pH of 6.8) was conducted to assess the microorganism's ability to ferment sugars and produce hydrogen sulfide (H_2S). The acidic byproducts of fermentation resulted in a yellow color, while alkaline byproducts yielded a red color. The formation of H_2S was indicated by a black color (22).

2.4.6. Citrate utilization test

The Citrate Agar (5 g/L NaCl, 0.2 g/L $MgSO_4$,1 g/L $NH_4H_2PO_4$, 1 g/L K_2HPO_4, 2 g/L sodium citrate, 0.08 g/L bromothymol blue, 15 g/L agar (15 grams) at a pH of 6.8). The citrate utilization test was used to detect the ability of an organism to utilize citrate as the sole carbon source for its growth (21).

2.4.7. Catalase test

A small amount of culture was placed over a clean slide. A drop of 3% hydrogen peroxide was placed over the culture and observed for effervescence. The effervescence production showed the organism's ability to produce the enzyme, catalase (22).

2.4.8. Oxidase test

Cytochrome oxidase in mitochondria and aerobic bacteria function to reduce molecular oxygen to yield water and generate energy by the formation of a transmembrane electrochemical gradient that drives ATP synthesis and other energy-demanding processes in the cells. Thus, the oxidase test was conducted to determine the presence of cytochrome c oxidase, an enzyme involved in the electron transport chain of certain bacteria. A small amount of culture was placed over a clean slide. Then a drop of oxidase reagent was added, and a purple or dark-blue color change developed within 10-30 seconds. This indicated a positive result (presence of oxidase). A lack of color change or a light pink coloration was considered a negative result (22).

2.4.9. Carbohydrate fermentation test

A carbohydrate fermentation medium (10 g/L peptone, 1 g/L beef extract, 5 g/L NaCl, 0.018 g/L phenol red) were dispensed 5 to 7 mL (or the volume required to submerge the Durham tube) of the broth into test tubes. It was ensured that each Durham tube was fully submerged without any trapped air bubbles. Then, the caps or cotton plugs were loosely secured and autoclaved the tubes for 15 minutes at 121°C and 15 lb pressure. Gas production along, with pH decrease were shown by a color change in the medium, which indicated positive carbohydrate fermentation. If there was no gas production or pH change, it suggested negative fermentation of the tested carbohydrate by the microorganism (22).

2.5. Isolation and 16S rRNA Identification of Bacterial Isolates

DNA was isolated for 16S rRNA sequencing, as this gene was a highly conserved region found in all the bacteria and archaea genomes. By isolating the DNA, it was ensured that we were working with the complete genetic material of an organism, allowing for the amplification, sequencing, and detailed analysis of the 16S rRNA gene. Sequencing this gene

allowed for the identification and classification of microorganisms, microbial diversity analysis for taxonomic identification, and phylogenetic analysis (23).

The isolates were grown on MRS broth till they reached the log phase (18 hours-old cultures). The cells were centrifuged at 5,000xg for 15 minutes. Extraction of DNA was performed using the HiMedia MB505 (HiPurA Bacterial Genomic DNA Purification) kit. In 200 µL of lysozyme solution, a loopful of the culture was suspended (45 mg/mL, i.e., 2.115 x 10^6 units/mL), and the suspension was incubated at 37°C for 30 minutes. RNase A solution (20 mg/mL) was added and incubated at room temperature for two minutes. 20µL of Proteinase K solution (20 mg/mL) was added, followed by 200µL of the lysis solution, vertexing the entire mixture, and incubated for ten minutes at 55°C. DNA was precipitated by the addition of 200µL of chilled 100% ethanol. The mixture was vortexed and the lysate was loaded onto the HiElute Miniprep Spin Column (HiMedia) as well as centrifugation was done at 6,500xg for three minutes. The flow-through liquid was discarded and 500µL of prewash solution was loaded into the spin column. This step was repeated three times. Lastly, the spin column was centrifuged for one minute at a maximum speed of 10,000xg to dry the column. The spin column was positioned on a fresh collection tube and 200µL of Elution Buffer was carefully loaded directly into column. For five minutes, the column was incubated at room temperature. The column was centrifuged at 6,500xg for 2 minutes to extract the DNA.

A UV spectrophotometer was employed to determine the DNA concentration (Shimadzu Corporation A11454806498). The isolated DNA was amplified for the 16S rRNA region by Polymerase Chain Reaction (PCR) utilizing a Biometra thermal cycler (T-Personal 48). A PCR reaction mix (procured from GeNei) consisting of 2.5µL of 10X buffer, 1µL of primers, 2.5µL of 2.5mM of each dNTP, 1µL template DNA as well as 2.5 U of Taq DNA polymerase, and nuclease-free water was prepared. PCR amplification cycle was as follows: one cycle of 5 minutes at 94°C, 35 cycles of 1 minute at 94°C, cooling for 1 minute at 50°C, two minutes at 72°C, as well as an additional cycle of 7 minutes at 72°C.

PCR was conducted using specific forward and reverse primers targeting the 16S rRNA gene (21). This amplification step facilitated the generation of multiple copies of the 16S rRNA gene, preparing the DNA for sequencing (23). The amplified PCR product was loaded onto a 1% agarose (HiMedia) gel to confirm successful amplification. Electrophoresis was carried out using Tris-Acetate-EDTA (TAE) buffer, and DNA bands were visualized with ethidium bromide staining. The PCR product was purified using manufactured protocol from the AxyPrep PCR Clean-up Kit (Axygen, AP-PCR-50).

2.6. Sequence Similarity and Phylogenetic Analysis

The purified DNA was used for sequencing (Applied Biosystems 3730xl DNA Analyzer, USA), along the chromatogram obtained was analyzed. The NCBI's (National Center for Biotechnology Information) nucleotide BLAST (Basic Local Alignment Search Tool) capability was employed to evaluate the DNA sequences. The consensus sequence was compared for similarity in the NCBI GenBank database using BLAST analysis (24). The Maximum Likelihood method was employed alongside the Kimura 2-parameter model to understand the evolutionary relationships among the analyzed taxa (25). The percentage of times the associated taxa clustered together in the bootstrap test (1,000 replicates) was indicated beside the branches. It depicted the evolutionary links among the taxa. Branches supported by less than 50% of bootstrap replicates were condensed (26,27).

Initial trees were generated using Neighbor-Join and BioNJ algorithms applied to pairwise distance data estimated through the Maximum Composite Likelihood (MCL) approach to initiate the analysis. The topology with the highest log likelihood value was selected. This analysis involved 11 nucleotide sequences, encompassing codon positions first, second, third, and noncoding regions. Positions containing gaps or missing data were excluded, resulting in a final dataset of 1434 positions. The evolutionary analyses were conducted using MEGA7 software. MEGA is a molecular evolutionary genetics analysis (MEGA) software program were used to generate phylogenetic trees. Bootstrap analysis was a method used to estimate the statistical reliability of clades in phylogenetic trees. The bootstrap value was the proportion of replicate phylogenies that recovered a particular clade from the original phylogeny (26,28).

3. RESULTS AND DISCUSSION

3.1. Proximate Analysis of Traditional Yogurts and Commercial Yogurt

In the present study, both the traditional yogurts exhibited reduced energy, carbohydrate, and fat content compared to commercial yogurt. This is indicating that indicating a potentially healthier nutritional profile for calorie-conscious consumers. In terms of protein content, both traditional yogurts exhibited higher levels, while commercial yogurt had lower protein levels. Moisture and ash content were similar among the traditional yogurt samples, while commercial yogurt exhibited higher ash content. Fiber content was negligible across all the samples. Table 1 indicated that all yogurt types contain very low fiber, below 0.2 grams/100 grams. Total solids were present at concentrations of 55.95% and 52.95% in *T. indica* L. derived traditional yogurt and *C. annuum* L. derived traditional yogurt, while commercial yogurt demonstarted a level of 52.62% (Table 1).

The traditional yogurt samples yielded lesser energy, and contained lesser carbohydrates, and total fat but more proteins than commercial yogurt which are beneficial for obese as well as diabetic individuals. Other parameters such as moisture, fiber, and total solids were the same among the three samples. Titratable acidity was higher in commercial yogurt

compared to traditional yogurts. This results were in alignment with study by Katidi et al. (29) which showed that the carbohydrate, fat and protein contents were 1–12 grams/100 grams, 0–20 grams/100 grams and 3.3–11 grams/100 grams, respectively. According to Saeed (30) energy content was 85.09–104.75 Kcal/100 grams, moisture was 79-80%, ash content was 0.28–0.95% and titrable acidity was 0.9-1.81%. Kowaleski et al. (31) showed that increasing milk total solids from 16 to 23 grams/100 grams improved the growth of *Lactobacillus bulgaricus* where counts of this organism after 240 minutes in 23% total solids is the highest.

3.2. Safety and Quality Profile of Traditional Yogurts and Commercial Yogurt

Regarding sensory characteristics, all the yogurt samples exhibited white color, smooth appearance, thick and creamy texture, and a subtle combination of sour and sweet taste, with a pH range of 4-5. The variations in yogurt color were attributed to the different biochemical activities of yogurt microorganisms, which transformed the casein complex from a micellar to a dispersed state. The consistency corresponded to textural and rheological measurements (32). The yogurt samples complied with Food and Drug Administration specifications, which stated that yoghurt should have a maximum pH of 4.5. The low pH value was the result of the amount of lactic acid produced from the fermentation of milk lactose by bacteria. Due to the low pH value, calcium would be converted to its ionic form, making it highly bioavailable for intestinal absorption. Also, the inhibitory effect of dietary phytic acid on calcium bioavailability would be reduced by the low pH value. The low pH value of yogurts also inhibited the growth of pathogens present in the yoghurts (30). However, when assessing the microbial safety, *Coliforms* such as *E. coli* was detected in the commercial yogurt, while both traditional yogurts showed the absence of these harmful bacteria. Fungi was absent in all the samples (Table 2).

The safety and quality profiles of all the three samples showed the same characteristics. However, *E. coli* and *coliforms* were present in the commercial yogurt sample. Poor hand washing, improper cleaning of equipment, and contaminated surfaces can contribute to introduction of *E. coli* and *coliforms*. *E. coli* in yoghurt brands is a serious health concern, as it can have negative implications for consumers. This poses a serious health risk to consumers, as these bacteria can cause severe infections like food poisoning and septicemia. The isolated *E. coli* resisted various antimicrobial agents, indicating a potential public health concern.

The study highlighted the importance of proper hygiene and sanitation in yoghurt production to prevent contamination with harmful pathogens like *E. coli*. This highlighted the need for improved quality control and sanitation measures in yogurt production to ensure consumer safety (33). The absence of *Coliforms* in the traditional yogurt samples could be due to specific strains of *Lactobacillus* species present in *T. indica* L. and *C. annuum* L. These *Lactobacillus* strains exert antimicrobial properties, thus, effectively suppressing the growth of pathogenic bacteria. This competitive inhibition or antimicrobial activity of the *Lactobacillus* strains could be one of the key reasons behind the absence of *E. coli* and *coliforms* in the traditional yogurt samples (34). The findings highlighted the potential superiority of traditional yogurts, particularly in terms of microbial safety, making them a preferable choice for consumers.

3.3. Biochemical Characteristics of Microbial Isolates from Yogurt Samples

Regarding their general characteristics, all three types of isolates grown on MRS agar exhibited tiny and round growth with a creamy, white-colored surface. Gram staining for all isolates showed a positive result, and the colony-forming units per milliliter (CFU/mL) were too numerous to count (TNTC), indicating abundant growth. The Indole test, Citrate utilization test, Methyl red test, Urease test, Triple Sugar Iron test, Voges-Proskauer test, Catalase test, and Oxidase test were all negative across all the three types of yogurts, except for the oxidase test, which showed a positive result in the *C. annuum* L. traditional yogurt isolate. Glucose and mannitol fermentation were positive in all the types of yogurts. *Lactobacillus* bacteria converted glucose and other carbohydrates into lactic acid through fermentation. This indicated that *Lactobacillus* species was present in the sample. Sucrose and fructose fermentation were positive in *T. indica* L. and *C. annuum* L. traditional yogurts, while commercial yogurt showed mixed results with sucrose fermentation. Maltose fermentation was observed only in the commercial yogurt isolate (Table 3).

Table 1. Proximate analysis of traditional yogurts and commercial yogurt

Parameters	*T. indica* L. Traditional yogurt	*C. annuum* L. Traditional yogurt	Commercial yogurt
Energy (Kcal/100 grams)	60.51	61.91	90.91
Carbohydrate (grams/100 grams)	3.23	3.83	9.90
Total fat (grams/100 grams)	3.80	3.50	7.80
Protein (grams/100 grams)	8.20	8.76	6.21
Moisture (%)	89	88	89
Ash (%)	0.7	0.91	1.21
Fiber (grams/100 grams)	<0.2	<0.2	<0.2
Total solids (%)	55.95	52.95	52.62
Titratable acidity (%)	0.31	0.28	0.58

Table 2. Safety and quality profile of traditional yogurts and commercial yogurt samples

Parameters	*T. indica* L. Traditional Yogurt	*C. annuum* L. Traditional Yogurt	Commercial Yogurt
Sensory characteristics			
Color	White	White	White
Appearance	Smooth	Smooth	Smooth
Texture	Thick, creamy	Thick, creamy	Thick, creamy
Taste	Subtle sour, sweet	Subtle sour, sweet	Subtle sour, sweet
pH	4-5	4-5	4-5
E. coli	Absent	Absent	Present
Coliform	Absent	Absent	Present
Fungi	Absent	Absent	Absent

Table 3. Biochemical characteristics of microbial isolates from yogurt samples

Parameters	*T. indica* L. Traditional Yogurt	*C. annuum* L. Traditional Yogurt	Commercial Yogurt
General Characteristics			
MRS agar	Tiny	Tiny	Tiny, round
Color	White	White	White
Surface	Creamy	Creamy	Smooth
Gram staining	+	+	+
CFU/mL	TNTC	TNTC	TNTC
Biochemical Characteristics			
Indole test	-	-	-
Methyl red test	-	-	-
Voges-Proskauer test	-	-	-
Urease test	-	-	-
Triple sugar iron test	-	-	-
Citrate utilization test	-	-	-
Catalase test	-	-	-
Oxidase test	-	+	-
Carbohydrate fermentation			
Glucose	+	+	+
Sucrose	+	+	+/-
Fructose	+	+	+/-
Maltose	-	-	+
Mannitol	+	+	+
Expected Microorganisms	*Lactobacillus* species	*Lactobacillus* species	*Lactobacillus* species

+: Answers the test; -: Do not answer the test; * TNTC: too-numerous-to-count

The biochemical characteristics were almost the same for all the yogurt samples. *C. annulus* L. showed a positive oxidase test, which indicated the presence of a functioning electron transport chain in the *Lactobacillus* species present in yogurt samples fermented by *C. annulus* L. Glucose, sucrose, fructose and mannitol except maltose were fermented by the home-made yogurt samples. On the contrary, *Lactobacillus* species were present in commercial yogurt fermented glucose, maltose, and mannitol, while ambiguous results were obtained for sucrose and fructose. The fermentation test helped to identify the bacteria. These findings suggested both similarities and differences among the microbial isolates from these yogurt samples in terms of their biochemical characteristics and carbohydrate fermentation, potentially indicating distinct microbial compositions in these yogurt types.

3.4. Authentication and Identification of *Lactobacillus* species Isolates

Identifying of *Lactobacillus* at species and strain level involved a combination of physiological and biochemical characteristics, as well as molecular techniques for accurate authentication. The microbial isolates obtained from yogurt samples were identified using the 16S rRNA gene sequencing technique, and amplified PCR products were purified and sequenced. Similarity sequence search for selected *Lactobacillus* isolates (*T. indica* L. traditional yogurt isolate, *C. annuum* L. traditional yogurt isolate, Commercial yogurt isolate) (NCBI, BLAST) revealed 99% homology with the available sequence of *L. acidophilus* and *L. plantarum*. The phylogenetic trees, which are graphical representations of the evolutionary relationships among different species or genes, in Figure 3 illustrate the relatedness of these isolates to the sequences retrieved from the database. Sample 1 illustrated that the *L. acidophilus* isolates closely clustered with reference strains NR 117812.1, NR 117062.1, NR 113638.1, and NR 043182.1, all showing robust 100% bootstrap support. This close clustering strongly suggested that the isolate shared a high degree of similarity with the established *L. acidophilus* strain.

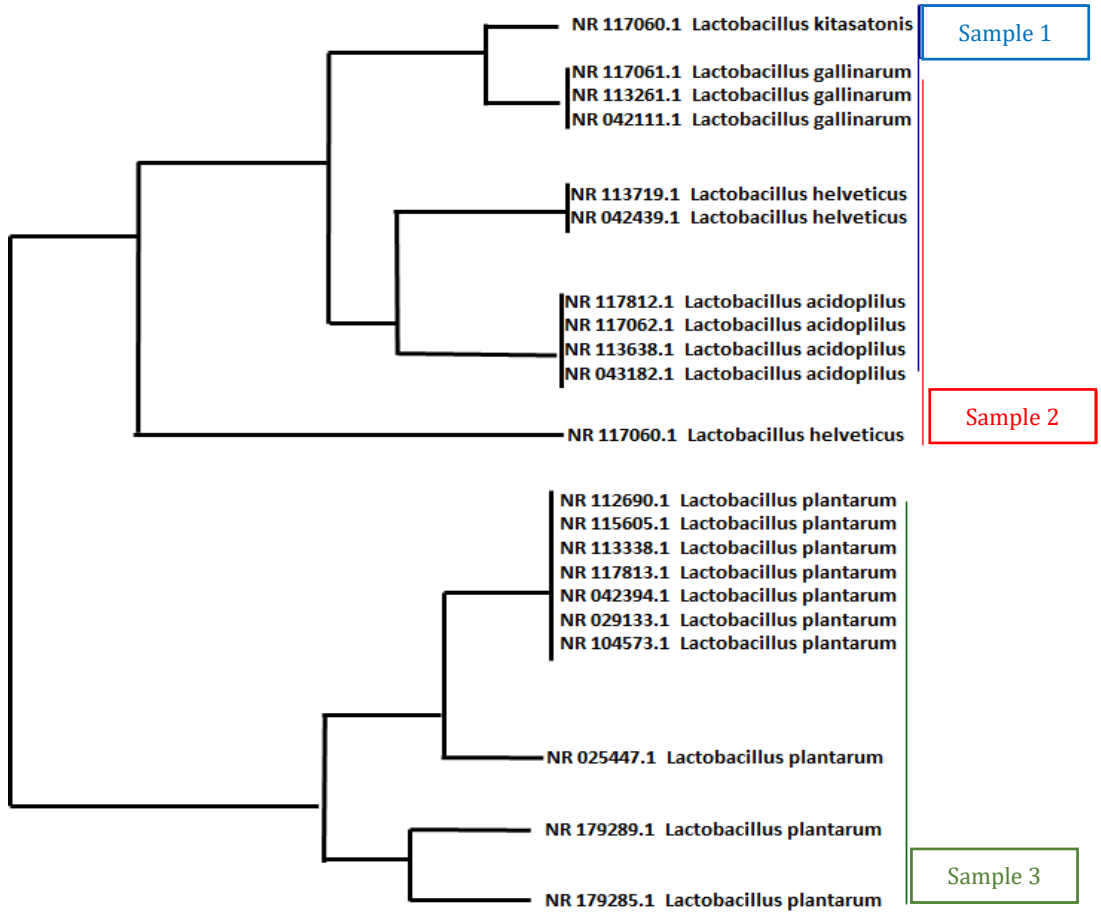

Figure 3. Molecular Phylogenetic analysis of *Lactobacillus* species from *T. indica* L. (sample 1) and *C. annuum* L. (Sample 2) traditional and commercial (Sample 3) yogurt microbial isolates by Maximum Likelihood Method.

Therefore, the *T. indica* L. traditional yogurt isolate was confirmed to contain *L. acidophilus*. Sample 2, the *L. acidophilus* isolates formed a tight cluster with reference strains NR 117062.1, NR 113638.1, NR 117812.1, and NR 043182.1 with 100% bootstrap support. This close grouping indicated significant similarity between the isolated strain and known reference strains. Thus, the *C. annuum* L. traditional yogurt isolate was identified to have *L. acidophilus*. Sample 3 demonstrated that the *L. plantarum* isolates clustered closely with reference strains NR 112690.1, NR 115605.1, NR 113338.1, and NR 117813.1, showing a strong 96% bootstrap support. This clustering strongly suggested a high resemblance between these strains thus the commercial yogurt isolate was found to be *L. plantarum* strains.

Dairy products are widely recognized as effective carriers for introducing beneficial probiotic bacteria into the human digestive system. Presently, there is a significant market demand for probiotic products. During the fermentation process, lactose in yogurt gets broken down, making it easier for lactose-intolerant individuals to consume without experiencing diarrhea, flatulence, and other symptoms associated with this condition. The breakdown of lactose into lactic acid during fermentation reduces the digestive challenges for those who have difficulty digesting lactose, enabling them to enjoy yogurt without experiencing adverse effects (35).

In contrast to homemade or traditional yogurts, commercial yogurt often contains food additives and preservatives that can have diverse consequences, depending on the additives used. Food additives like maltodextrin and some non-caloric artificial sweeteners present in commercial yogurt have shown harmful effects on gut health. Studies indicate that certain artificial sweeteners, such as saccharin, can lead to dysbiosis in the gut microbiota, potentially contributing to glucose intolerance (36). These additives compromise the health benefits of probiotics, which are the primary reason for consuming commercial probiotic yogurt. In contrast, homemade traditional yogurt with probiotics appears to be preferable.

4. CONCLUSIONS

The traditional yogurt samples yielded lesser energy and contained lesser carbohydrate and total fat but more proteins than the commercial yogurt, which is beneficial for obese as well as diabetic individuals. The safety and quality profiles of

all the three samples showed the same characteristics. However, *E. coli* and *coliforms* were present in the commercial yogurt sample. The biochemical characteristics were almost the same for all the yogurt samples. *C. annulus* L. showed a positive oxidase test, which indicated the presence of a functioning electron transport chain in the Lactobacillus species present in yogurt samples fermented by *C. annulus* L. Glucose, sucrose, fructose, and mannitol were fermented by the home-made yogurt samples except for maltose. The fermentation test helped to identify the bacteria. The specific strains of *Lactobacillus* species present in both traditional yogurts contributed to their antimicrobial properties. In summary, the findings suggest that traditional yogurts, with their distinctive characteristics and potential health benefits, could be considered the preferable choice for consumers seeking a wholesome and safe yogurt option. Further, in vivo studies are being planned to elucidate its human health benefits for specific diseases.

Author contributions: ST, SS: Conceptualization, methodology, validation, resources, data curation, funding acquisition. SS, EB: software. ST, SS, EB, GV, MP: formal analysis, writing—original draft preparation, writing—review and editing, visualization, supervision. SS, GV: project administration. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Shri N.P.V Ramasamy Udayar Research Fellowship, grant number U02M210724 and the APC was funded by ST (author). The funding received was sufficient to conduct the research.

Acknowledgements: Authors wish to thank SRIHER for providing necessary infrastructure to conduct the necessary.

Ethics statement: Since the study did not involve animals or human beings, it did not require ethics approval.

Conflict of interest: None.

REFERENCES

1. Behera SS, Panda SK. Ethnic and industrial probiotic foods and beverages: Efficacy and acceptance. Current Opinion in Food Science. 2020;32:29-36. https://doi.org/10.1016/j.cofs.2020.01.006
2. Bintsis T, Papademas P. The evolution of fermented milks, from artisanal to industrial products: A critical review. Fermentation. 2022;8(12):679. https://doi.org/10.3390/fermentation8120679
3. Bangar SP, Suri S, Trif M, Ozogul F. Organic acids production from lactic acid bacteria: A preservation approach. Food Biosci. 2022;46:101615. https://doi.org/10.1016/j.fbio.2022.101615
4. Paul C, Mishu ID, Miah MI, Bari ML, Rahman SR, Malek MA. Isolation, identification and probiotic potential of lactic acid bacteria and yeasts from commercial yogurt and homemade non-dairy fermented food "KANJI". Int J Gastron Food Sci. 2023;34:100787. https://doi.org/10.1016/j.ijgfs.2023.100787
5. Arslan S, Akagündüz D, Cebecioglu R, Kul A, Çatal T. Yoghurt production using pinecone and investigation of the effect of the produced yogurt on ECV304 cell line. Int J Life Sci Biotechnol. 2021;5(1):29-41. https://doi.org/10.38001/ijlsb.962106
6. Bostan K, Unver Alcay A, Yalçin S, Eren Vapur U, Nizamlioglu M. Identification and characterization of lactic acid bacteria isolated from traditional cone yoghurt. Food Sci Biotechnol. 2017;26(6):1625-32. https://doi.org/10.1007/s10068-017-0222-z
7. da Silva JF, Miranda RF, Costa HR, Lima CM, Verruck S, Barbosa EA, et al. Bilayer goat's milk yogurt with tamarind (*Tamarindus indica* L.) and wild passion fruit (*Passiflora cincinnata* Mast) jam: Characterization and acceptability. Res Soc Dev. 2020;9(9):e46996139. https://doi.org/10.33448/rsd-v9i9.6139
8. Abiraami R, Palanidorai R, Pugazhenthi TR, Prabu M. Physicochemical and sensory evaluation of functional yoghurt enriched with tamarind seed kernel powder. J Pharmacogn Phytochem. 2021;10:1852-5. Available from: https://www.phytojournal.com/archives/2021/vol10issue1/PartZ/10-1-298-936.pdf
9. Madhogaria B, Bhowmik P, Kundu A. Correlation between human gut microbiome and diseases. Infectious Medicine. 2022;1(3):180-91. https://doi.org/10.1016/j.imj.2022.08.004
10. Abiraami R, Dorai RP, Pugazhenthi TR, Prabu M. Development of functional milk lollies utilizing lyophilized tamarind seed kernel powder. Indian J Vet Anim Sci Res. 2022;51(4):26-34. Available from: https://epubs.icar.org.in/index.php/IJVASR/article/view/129572
11. Mokbel SM, Abdeldaiem AM. Production a novel flavoured-yoghurt drink using tamarind extract. Ismailia J Dairy Sci Technol. 2022;9(1):1-10. https://doi.org/10.21608/ijds.2022.290549
12. Olukotun GB, Salami SA, Okon IJ, Ahmadu JH, Ajibulu OO, Bello Z. Assessment of the effects of back sloping on some starter culture strains and the organoleptic qualities of their yoghurt products. Asian Food Sci J. 2021;20(9):29–36. https://doi.org/10.9734/afsj/2021/v20i930340
13. Latimer GW. Official Methods of Analysis: 22nd Edition. AOAC Publications. 2023, https://doi.org/10.1093/9780197610145.002.001
14. Grasso N, Alonso-Miravalles L, O'Mahony JA. Composition, physicochemical and sensorial properties of commercial plant-based yogurts. Foods. 2020;9(3):252. https://doi.org/10.3390/foods9030252
15. Malomo AA, Ogunjemilusi M, Ibukun OF, Ogechukwu EJ. Microbiological characteristics, proximate composition and sensory properties of plant-based set yoghurt analogue produced from blends of maize-soybean. Food

Science and Applied Biotechnology. 2024;7(1):58-66. https://doi.org/10.30721/fsab2024.v7.i1.299

16. Yang SY, Yoon KS. Effect of probiotic lactic acid bacteria (LAB) on the quality and safety of Greek yogurt. Foods. 2022;11(23):3799. https://doi.org/10.3390/foods11233799
17. Aktar T. Physicochemical and sensory characterisation of different yoghurt production methods. Int Dairy J. 2022;125:105245. https://doi.org/10.1016/j.idairyj.2021.105245
18. Renschler MA, Wyatt A, Anene N, Robinson-Hill R, Pickerill ES, Fox NE, et al. Using nitrous acid-modified de Man, Rogosa, and Sharpe medium to selectively isolate and culture lactic acid bacteria from dairy foods. J Dairy Sci. 2020;103(2):1215-22. https://doi.org/10.3168/jds.2019-17041
19. Nemati V, Hashempour-baltork F, Mirza Alizadeh AM, Varzakas T. Production of traditional torba yogurt using lactic acid bacteria isolated from fermented vegetables: microbiological, physicochemical and sensory properties. J Agric Food Res. 2023;14:100850. https://doi.org/10.1016/j.jafr.2023.100850
20. Rahman MS, Emon DD, Nupur AH, Mazumder MA, Iqbal A, Alim MA. Isolation and characterisation of probiotic lactic acid bacteria from local yogurt and development of inulin-based synbiotic yogurt with the isolated bacteria. Appl Food Res. 2024;4(2):100457. https://doi.org/10.1016/j.afres.2024.100457
21. Bhutia MO, Thapa N, Tamang JP. Molecular characterization of bacteria, detection of enterotoxin genes, and screening of antibiotic susceptibility patterns in traditionally processed meat products of Sikkim, India. Front Microbiol. 2021;11:599606. https://doi.org/10.3389/fmicb.2020.599606
22. Hasani I, Sepahi AA, Shoeibi S, Aghdash GA. Isolation and identification of probiotic bacteria from Iranian traditional cheese produced by lamb or kid abomasum microorganisms. J Food Saf Hyg. 2020;6(4):221-30. https://doi.org/10.18502/jfsh.v6i4.7567
23. Rizal NS, Neoh HM, Ramli R, Hanafiah A, Samat MN, Tan TL, et al. Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: Perspectives from a middle-income country. Diagnostics. 2020;10(10):816. https://doi.org/10.3390/diagnostics10100816
24. Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S. Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clin Microbiol Rev. 2020;33(4):e00053-19. http://doi.org/10.1128/CMR.00053-19
25. Zou Y, Zhang Z, Zeng Y, Hu H, Hao Y, Huang S, Li B. Common methods for phylogenetic tree construction and their implementation in R. Bioengineering. 2024;11(5):480. https://doi.org/10.3390/bioengineering11050480
26. Mahadani AK, Awasthi S, Sanyal G, Bhattacharjee P, Pippal S. Indel-K2P: A modified Kimura 2 Parameters (K2P) model to incorporate insertion and deletion (Indel) information in phylogenetic analysis. Cyber-Physical Systems. 2022;8(1):32-44. https://doi.org/10.1080/23335777.2021.1879274.
27. Ben Ayed A, Akrout I, Albert Q, Greff S, Simmler C, Armengaud J, et al. Biotransformation of the fluoroquinolone, levofloxacin, by the white-rot fungus *Coriolopsis gallica*. J Fungi. 2022;8(9):965. https://doi.org/10.3390/jof8090965
28. Russo CA, Selvatti AP. Bootstrap and rogue identification tests for phylogenetic analyses. Mol Biol Evol. 2018;35(9):2327-33. https://doi.org/10.1093/molbev/msy118
29. Katidi A, Xypolitaki K, Vlassopoulos A, Kapsokefalou M. Nutritional quality of plant-based meat and dairy imitation products and comparison with animal-based counterparts. Nutrients. 2023;15(2):401. https://doi.org/10.3390/nu15020401
30. Saeed M. Physicochemical analysis of yogurt fortified with *Moringa oleifera* leaf powder. Authorea Preprints. 2020. https://doi.org/10.22541/au.160010855.57785840
31. Kowaleski J, Quast LB, Steffens J, Lovato F, dos Santos LR, da Silva SZ, et al. Functional yogurt with strawberries and chia seeds. Food Bioscience. 2020;37:100726. https://doi.org/10.1016/j.fbio.2020.100726
32. Ilić N, Belović M, Memiši N, Pestorić M, Škrobot D, Pezo L, et al. Rheological properties and sensory profile of yoghurt produced with novel combination of probiotic cultures. Foods. 2024;13(19):3021. https://doi.org/10.3390/foods13193021
33. Chukwuemeka ON, Owhonka A, Awari VG, Teedum D. Isolation and identification of *Escherichia coli* (*E. coli*) in home-made and commercially produced yoghurt sold in port Harcourt. J Adv Microbiol. 2024;5(2):10-6. https://www.microbiojournal.com/archives/2024.v5.i2.A.157
34. Soltani N, Abbasi S, Baghaeifar S, Taheri E, Jadid MFS, Emami P, et al. Antibacterial and antibiofilm activity of Lactobacillus strains secretome and extraction against *Escherichia coli* isolated from urinary tract infection. Biotechnol Rep (Amst). 2022;36:e00760. https://doi.org/10.1016/j.btre.2022.e00760
35. Yang S, Yan D, Zou Y, Mu D, Li X, Shi H, et al. Fermentation temperature affects yogurt quality: A metabolomics study. Food Biosci. 2021;42:101104. https://doi.org/10.1016/j.fbio.2021.101104
36. Illikoud N, Mantel M, Rolli-Derkinderen M, Gagnaire V, Jan G. Dairy starters and fermented dairy products modulate gut mucosal immunity. Immunol Lett. 2022;251-252:91-102. https://doi.org/10.1016/j.imlet.2022.11.002