Assessment of Probiotic Properties and Consumer Acceptability of Yogurt Made from Commercial Milk using Bacterial Isolates from *NONO*

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Abstract— *Nono is a traditionally fermented milk product commonly consumed in parts of Nigeria. It undergoes spontaneous fermentation by lactic acid bacteria (LAB), conferring potential probiotic properties. This study isolated and identified LAB from Nono and assessed their suitability as novel starter cultures for yogurt production using commercial milk. Three LAB isolates were obtained from Nono samples on selective media. They were Gram-positive rods, catalase-negative, with ability to ferment various sugars. The isolate with the greatest antimicrobial activity against Escherichia coli and Staphylococcus aureus was molecularly identified as Lactobacillus fermentum. Set-type yogurt was produced at laboratory scale by inoculating reconstituted commercial milk (14% total solids) with 2% of commercial starter culture (Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus); 2% L. fermentum; and 1% each of commercial starter and L. fermentum. The probiotic, physicochemical, sensory and storage properties were analyzed. L. fermentum-containing yogurts had lower pH and higher titratable acidity than the control yogurt. Proximate composition was similar across samples. Sensory evaluation showed comparable consumer acceptability, with slight preference for the control sample. Yogurt with L. fermentum maintained higher viable LAB during storage at refrigeration temperature for 28 days. The findings demonstrate the potential for using LAB isolates from traditionally fermented foods like Nono as novel starter cultures in yogurt manufacture. This can promote product diversification and valorization of indigenous fermentation practices*.

***Keywords— Nono, lactic acid bacteria, yogurt, probiotics, starter culture, consumer acceptability*.**

I. INTRODUCTION

Probiotics are live microbial food supplements that confer health benefits on the host when consumed in adequate amounts (Hill *et al.*, 2014). Fermented foods are regarded as important probiotic carriers, with yogurt being the foremost dairy product acclaimed for its probiotic properties (Granato *et al.,* 2010). Yogurt popularity has surged in recent years due to associated nutritional and therapeutic advantages (Hekmat and Reid, 2006). Its industrial production involves fermentation of milk by starter cultures consisting principally of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Sfakianakis and Tzia, 2014). These bacteria impart yogurt's characteristic texture, flavor and acidity by producing lactic acid from lactose fermentation (Gonzalez-Gonzalez *et al.*, 2011).

Besides the traditional yogurt starters, other lactic acid bacteria (LAB) can also be incorporated as adjunct cultures to diversify yogurt products. For instance, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* spp. are often used as probiotic enrichments in commercial yogurts to enhance health attributes (Donkor *et al.,* 2007). A growing consumer preference for natural, minimally processed foods has fueled interest in using novel LAB strains isolated from traditional fermented products as starter cultures (Bourdichon *et al.,* 2012). Such strains possess inherent robustness and diverse enzymatic activities tailored by years of adaptation to their natural niches. This can potentially improve yogurt functionality, quality and uniqueness (Leroy and De Vuyst, 2004).

Nono is a traditional fermented milk product widely consumed in Northern Nigeria for its nutritional value. It is produced by Fulani pastoralists through spontaneous fermentation, relying on back-slopping with previous batches to propagate the microbiota (Beukes *et al.,* 2001; Oguntoyinbo and Narbad, 2015). *Nono's* fermentation profile and microbial diversity have been elucidated in a few studies. Isolates obtained include *Lactobacillus delbrueckii, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc lactis, Leuconostoc mesenteroides* and *Pediococcus acidilactici* (Banwo *et al.,* 2020). *Nono* thus represents a promising reservoir of novel LAB that can be harnessed for functional starter culture development. This study therefore aimed to isolate and identify LAB from *Nono*, evaluate their probiotic properties, and assess the physicochemical qualities, consumer acceptability and stability of yogurt made by incorporating selected *Nono* isolates alongside conventional starter cultures in commercial milk fermentation.

II. MATERIALS AND METHODS

2.1 Sample Collection

Nono samples were purchased from a local producer in Gariki, Amansea, Anambra State, Nigeria and immediately transferred aseptically into sterile flasks. Commercial full cream powdered milk and conventional yogurt starter culture (Yogurmet, France) containing *L. bulgaricus* and *S. thermophilus* lyophilized direct-vat-set cultures were also obtained from Eke Awka Market, Awka, Anambra State, Nigeria.

2.2 Isolation and Identification of LAB from Nono

MRS (de Man, Rogosa and Sharpe) agar (pH 5.4) was used to selectively isolate *Lactobacillus* spp. from the *Nono* samples by anaerobic pour plate technique, while TOS-MUP (transgalactosylated oligosaccharides-mupirocin lithium salt) agar facilitated selective isolation of *Bifidobacterium* spp. based on the methodology described by Süle *et al.* (2014). Discrete colonies were randomly picked and purified by successive subculture on fresh media. Also, Isolates were screened by Gram staining, catalase and oxidase tests. Three presumptive LAB isolates that were Gram-positive rods, catalase-negative and oxidase-negative were selected for further characterization (Süle *et al.*, 2014). Their ability to ferment different sugars was assessed using inverted Durham tubes in peptone water with 1% sugar substrates. The isolate exhibiting greatest antibacterial activity was identified by 16S rDNA sequencing using genomic DNA extraction, PCR amplification with universal primers and Sanger sequencing (Magray *et al.,* 2020). The ~1500 bp 16S rRNA sequence obtained was matched against the NCBI GenBank database using BLASTn to determine closest phylogenetic relatives.

2.3 Preparation of Yogurt Samples

The powdered milk was reconstituted to 14% total solids based on standard methods (Suzanne, 2003) and pasteurized at 90^{o}C for 15 minutes. It was cooled to 42^{o}C and inoculated with different starter cultures as follows:

S - 2% commercial yogurt starter

SP - 2% *Lactobacillus* isolate from Nono

Mix - 1% commercial yogurt starter + 1% *Lactobacillus* isolate

Inoculated milk samples were incubated at 42^{o}C for 4 hours to achieve a titratable acidity of 0.9% lactic acid. The yogurts were then cooled to 4^{o}C and analyzed while fresh (day 0) as well as on days 14 and 28 of refrigeration storage. All experiments were performed in triplicate.

2.4 Assessment of Probiotic Properties of LAB Isolates

2.4.1 Antimicrobial Activity

The agar well-diffusion assay was used to evaluate cell-free supernatants of LAB isolates against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 12600 as described previously (Barefoot and Klaenhammer, 1983). Zones of inhibition were measured after 24h incubation.

2.4.2 Acid Tolerance

Isolates were grown in broth, harvested by centrifugation and resuspended in sterile phosphate buffered saline (pH 7.0) at 10x concentration. This cell suspension was inoculated (1% v/v) into simulated gastric juice (pepsin 3g/L in saline, pH 2.0) and viable counts were determined after 0, 1, 2 and 3h by plating dilutions on MRS agar (Shehata *et al.,* 2016).

2.4.3 Bile Tolerance

Similarly, washed isolate suspensions were inoculated into MRS broth containing 0.3% oxgall bile (HiMedia, India). Viable counts and optical density (625 nm) were monitored after 0, 1, 2 and 3h incubation to assess bile tolerance (Shehata *et al.,* 2016).

2.5 Analysis of Yogurt Quality

2.5.1 pH and Titratable Acidity

Yogurt samples were stirred with distilled water before measuring pH directly using a digital pH meter. Titratable acidity expressed as % lactic acid was determined by titrating yogurt samples with 0.1N NaOH using phenolphthalein indicator (AOAC, 2005; AOAC, 1990).

2.5.2 Viscosity

Viscosity (Pa.s) was measured using a Brookfield viscometer at 150 rpm and 25oC on fresh and stored yogurt samples.

2.5.3 Proximate Analysis

Moisture, fat, protein, ash and carbohydrate content of fresh yogurt samples were analyzed using standard AOAC methods (AOAC, 2005; AOAC, 1990). Total carbohydrate was estimated by difference.

2.5.4 Lactic Acid Content

Lactic acid concentration was determined spectrophotometrically using 0.2% $FeCl_3$ reagent and comparing absorbance at 390 nm against a standard curve of known lactic acid concentrations (Borshchevskaya *et al.,* 2016).

2.5.5 Sensory Evaluation

A 9-point hedonic scale test was conducted with 20 untrained panelists to evaluate the appearance, color, aroma, taste, texture and overall acceptability of fresh yogurt samples. The scales were anchored at 1 for extreme dislike and 9 for extreme like (Udezor, 2012).

2.6 Microbiological Analysis

2.6.1 Antibacterial Activity

Cell-free supernatants were prepared from yogurt samples and their ability to inhibit *Escherichia coli* ATCC 25922 was evaluated by agar well-diffusion assay on days 0, 14 and 28 of refrigeration storage.

2.6.2 Viability of Starter Cultures

Serial dilutions of yogurt samples were plated on MRS agar and incubated anaerobically at 37oC for 48 hours to determine viable LAB counts on days 0, 14 and 28 of storage.

2.7 Data Analysis

All data were analyzed by one-way ANOVA and Duncan's multiple range test using SPSS version 20.0. Mean values were considered significantly different at $p<0.05$.

III. RESULTS

3.1 Isolation and Identification of Probiotic Bacteria

A total of three lactic acid bacteria were isolated from *nono* samples on MRS agar. The isolates were characterized biochemically and identified as rod-shaped, Gram-positive, catalase-negative bacteria resembling Lactobacillus species (Table

1). Further identification of the isolates was done by 16S rDNA sequencing, which revealed isolate B to be *Lactobacillus fermentum* strain 17-6 (Figure 1).

TABLE 1
BIOCHEMICAL CHARACTERIZATION OF LACTIC ACID BACTERIA FROM *NONO*

Biochemical Tests	Isolate A	Isolate B	Isolate C
Gram stain	+	+	+
Cell morphology	Rod	Rod	Rod
Catalase reaction	-	-	-
Oxidase	+	+	+
Glucose fermentation	+	+	+
Gas from glucose	-	-	+
Lactose fermentation	+	+	+
Sucrose fermentation	+	+	+
Galactose fermentation Probable Organism	+ *Lactobacillus* sp.	+ *Lactobacillus* sp..	+ *Lactobacillus* sp.

Keys: (+) = positive test; (-) = negative test; Sp = Species

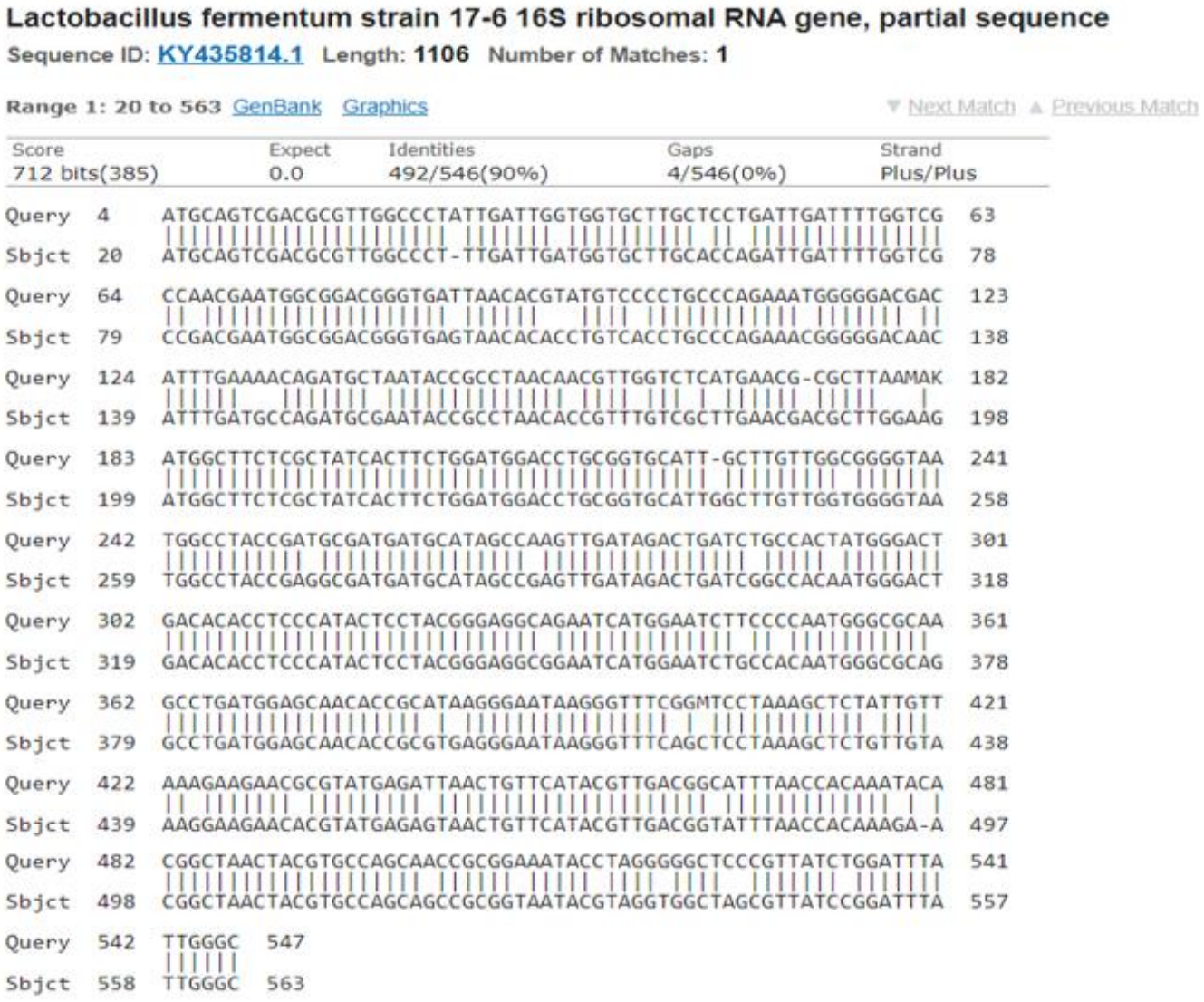

FIGURE 1: Gene Sequence of *Lactobacillus fermentum*

3.2 Assessment of Probiotic Properties

The three isolates were assessed for various probiotic characteristics including acid tolerance, bile tolerance and antimicrobial activity. Growth at different pH showed that all isolates were able to grow at pH 4.0 and pH 7.0, with maximum growth observed at pH 4.0 (Table 2). This indicates the isolates can survive the acidic conditions of the gastrointestinal tract. Additionally, the isolates demonstrated bile tolerance by growing on MRS agar supplemented with 0.3% oxgall bile, confirming their ability to withstand the physiological concentrations of bile salts (Table 3). Of the three isolates, isolate B (*L. fermentum*) exhibited the strongest antimicrobial activity against *E. coli* and *S. aureus*, with inhibition zone diameters of 20.64 ± 0.04mm and 18.50 ± 0.50mm respectively (Table 4). The antibacterial activity suggests the isolate's potential to inhibit pathogens and maintain gut health. Based on the probiotic characteristics demonstrated, isolate B (*L. fermentum*) was selected for further assessment in yogurt production.

TABLE 2
GROWTH OF LACTIC ACID AT DIFFERENT PH

Isolate codes	pH 2.5	pH 4.0	pH 7.0
A	+	++	++
B	++	+++	++
C	+	++	++

Keys: (+) = positive test

TABLE 3
SURVIVAL OF ISOLATES IN FRESH BOVINE BILE

Isolate Codes	Survival in Fresh Bovine Bile
A	+
B	+
C	+

TABLE 4
DIAMETER ZONES OF INHIBITION (mm) FOR ANTIMICROBIAL ACTIVITY OF PROBIOTIC ISOLATES AGAINST SELECTED PATHOGENS

Isolate Codes	*Escherichia coli*	*Staphylococcus aureus*
A	17.38 ± 0.10	16.67 ± 1.15
B	20.64 ± 0.04	18.50 ± 0.50
C	15.27 ± 0.25	12.47 ± 0.41

3.3 Physicochemical Properties of Yogurt

Yogurt was prepared using a commercial starter culture (S), *L. fermentum* isolate (SP) and a mix of both (mix). The physicochemical properties evaluated were pH, titratable acidity, viscosity and total solids (Table 5). Yogurt S had the highest viscosity (10.51 ± 0.01 Pa.s) and total solids (21.70%), while SP and mix had lower pH values (4.11 ± 0.01 and 4.26 ± 0.01 respectively) and higher titratable acidity indicating increased lactic acid production. This was confirmed by lactic acid quantification, where mix had the highest lactic acid concentration within 24hrs (Figure 2).

TABLE 5
PHYSICO-CHEMICAL EVALUATION OF YOGURT SAMPLES

Parameters	S	SP	Mix
Total solid (%)	21.70	18.90	20.08
pH	4.52 ± 0.03	4.11 ± 0.01	4.26 ± 0.01
Titratable Acidity	1.16 ± 0.02	1.10 ± 0.02	1.21 ± 0.02
Viscosity (Pa.s)	10.51 ± 0.01	8.21 ± 0.01	7.21 ± 0.01

Keys: S = Standard yogourmet; Sp = Lactobacillus fermentum; Mix = Mixture of standard yogurmet plus Lactobacillus fermentum

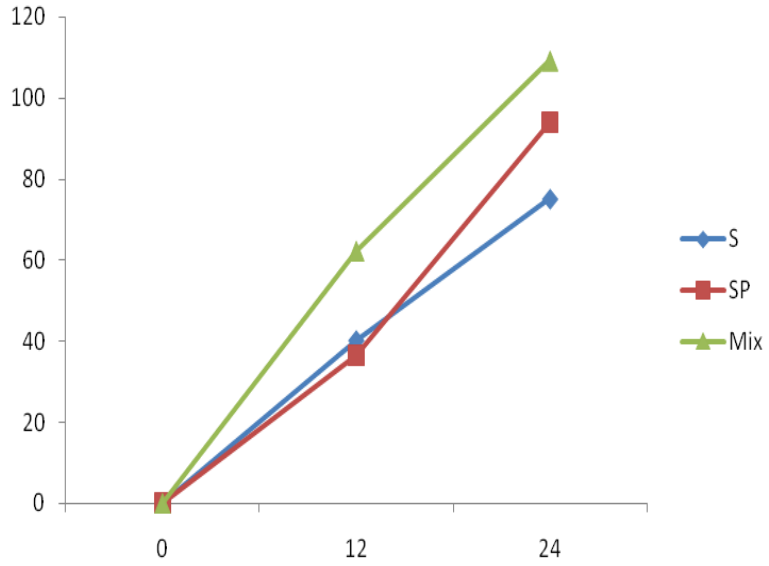

FIGURE 2: Lactic Acid Content of Yogurt Samples

Keys: S- standard yogurmet; SP- Lactobacillus fermentum; Mix- Mixture of standard yogurmet plus lactobacillusfermentum

3.4 Proximate Composition

The nutritional composition of the yogurts was analyzed in terms of moisture, ash, fat, protein, fiber and carbohydrates (Table 6). Yogurt SP had slightly higher moisture, ash, fat and protein compared to S and mix. Notably, SP had higher fiber (3.74%) but lower carbohydrates (5.73%) versus S and mix.

TABLE 6
PROXIMATE COMPOSITION OF YOGURT SAMPLES

Parameters (%)	Sample S	Sample SP	Mix
Moisture content	85	86	84
Ash	0.70	0.80	0.92
Crude fat	3.61	3.74	3.81
Crude protein	3.18	3.2	3.31
Crude fibre	0.51	0.53	0.49
Carbohydrate	7.00	5.73	7.47

Keys: S = Standard yogurmet; Sp = Lactobacillus fermentum; Mix = Mixture of standard yogurmet plus Lactobacillus fermentum

3.5 Sensory Evaluation

Sensory analysis of the yogurts indicated that S had the highest overall acceptability based on appearance, taste and mouthfeel (Table 7). However, there were no marked differences between the samples for aroma and color. This suggests that incorporation of the *L. fermentum* isolate did not adversely affect sensory properties.

TABLE 7
SENSORY EVALUATION OF YOGURT SAMPLES

Parameters	S	SP	Mix
Appearance	8.00	7.00	8.00
Aroma	7.00	7.00	7.00
Taste	8.00	8.50	7.00
Mouth-Feel	6.00	5.00	4.00
Overall Acceptability	8.00	7.00	7.00

Keys: S = Standard yogurmet; Sp = Lactobacillus fermentum; Mix = Mixture of standard yogurmet plus Lactobacillus fermentum

3.6 Storage Viability

The viability of probiotic bacteria and antimicrobial activity against *E. coli* was evaluated during refrigerated storage for 28 days (Tables 8 and 9). Although viable counts decreased over time in all samples, mix maintained the highest counts at day 14 (14.2 x 104 cfu/ml) and day 28 (10.8 x 104 cfu/ml) indicating better probiotic survival. Antimicrobial activity also declined with storage but was likewise highest in mix yogurt compared to S and SP.

TABLE 8
TOTAL VIABLE COUNT (X104) FOR LACTIC ACID BACTERIA FOR THE YOGHURT SAMPLES

Samples	Day 14	Day 28
S	9.2	6.2
SP	10.8	4.5
Mix	14.2	10.8

Keys: S = Standard yogurmet; Sp = Lactobacillus fermentum; Mix = Mixture of standard yogurmet plus Lactobacillus fermentum

IV. DISCUSSION

The present study isolated three lactic acid bacteria from traditionally fermented *Nono* milk, with one isolate identified as *Lactobacillus fermentum* based on 16S rRNA gene sequencing. *Lactobacillus fermentum* has previously been reported as one of the predominant LAB in Nigerian *Nono*, conferring its probiotic properties (Obi *et al.,* 2016). In agreement, our isolated *L. fermentum* strain exhibited good probiotic characteristics including acid and bile tolerance as well as antimicrobial activity against pathogenic *E. coli* and *S. aureus*. These properties enable probiotic bacteria to survive gastrointestinal transit and inhibit pathogens through the production of bacteriocins and organic acids (Granato *et al.,* 2010; Donkor *et al.,* 2007).

Yogurt manufactured by incorporating the *Nono*-derived *L. fermentum* alongside conventional starter cultures demonstrated enhanced physicochemical attributes compared to the control yogurt made solely with starters. Specifically, the *L. fermentum*-containing yogurt had lower pH and higher titratable acidity, indicating increased lactic acid production (Sfakianakis & Tzia, 2014). This was corroborated by direct lactic acid quantification. The higher acidity can extend yogurt's shelf life by suppressing spoilage microorganisms. Despite compositional differences, sensory analysis revealed comparable consumer acceptability across all yogurt samples. This suggests that the *L. fermentum* adjunct did not adversely affect flavor and texture attributes. Mild or no flavor defects upon adding probiotic cultures to yogurt have been documented before (Donkor *et al.,* 2007).

During refrigeration storage, the viability of probiotic bacteria decreased in all yogurts as expected, but viable counts remained significantly higher in the L. fermentum-supplemented yogurt compared to the control after 28 days. Enhanced starter culture survival indicates the robustness of the *Nono* isolate for maintaining yogurt functionality over time (Gonzalez-Gonzalez *et al.,* 2011). In summary, the indigenous *L. fermentum* strain isolated from traditionally fermented *Nono* demonstrated strong

probiotic properties that can be harnessed to develop novel functional starter cultures for yogurt production. Incorporating the isolate improved physicochemical and storage qualities without compromising sensory attributes.

V. CONCLUSION

The findings of this study demonstrate the potential of isolates obtained from traditionally fermented *Nono* to be developed as novel functional starter cultures for yogurt production. The *Lactobacillus fermentum* isolate showed good survival under conditions simulating the gastrointestinal tract, indicating its robust probiotic properties. Incorporation of this isolate into yogurt fermentation enhanced the physicochemical attributes by improving acidification compared to the control. Sensory evaluation revealed comparable consumer acceptability among yogurts produced with different starter combinations. Moreover, viable counts of probiotic bacteria were maintained at a higher level during refrigeration storage of yogurt with added *L. fermentum*. Overall, the results suggest that LAB from indigenous fermented foods like *Nono* have the capacity to be developed as adjunct starter cultures for diverse probiotic dairy products. This offers opportunities to valorize traditional fermentation practices and promote product innovation through strain diversification. Further research could optimize the *Nono* isolate's application in diverse dairy and non-dairy probiotic foods.

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