CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2025.066

Selection, identification, and application in yogurt fermentation of lactic acid bacteria isolated from guava (*Psidium guajava* L.) fruits

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Article info.

Received 14 Oct 2024
Revised 22 Dec 2024
Accepted 2 Jun 2025

Keywords

Antibacterial activity, guava fruit, isolation and identification, lactic acid bacteria, organic acid-producing capacity

ABSTRACT

Lactic acid bacteria (LAB) can be isolated from different natural sources such as plants, fruits, or vegetables. The organic acid-producing capacity and antibacterial activity of each LAB strain are highly dependent on its isolation source. In the study, LAB strains were isolated from guava (Psidium guajava L.), identified for organic acid production and antibacterial activity. Twenty LAB isolates showed rod, oval, or cocci shape, Gram-positive, catalase-negative, oxidase-negative, the ability to degrade $CaCO_3$, and could not produce indole from tryptophan. Especially, the strain TN2 produced the highest organic acid content (8.7±0.14 g/L) as well as exhibited the strongest antibacterial activity against Escherichia coli (E. coli) with the inhibition zone diameter of 12.33±2.08 mm. The bacteria strain TN2 was applied to ferment yogurt with 8% guava juice, at a milk and condensed milk ratio of 2:1, giving the best lactic acid content and sensory evaluation. The strain TN2 was identified as Lactococcus lactis strain according to the 16S rRNA gene sequence analysis and registered on GenBank with code number MN860000.1. This strain can be a potent alternative to commercialized LAB strains in fermented food products.

1. INTRODUCTION

Fermented foods, in which numerous carbohydrates are broken down by the presence of living probiotics, play a vital role in the human diet (Mulaw et al., 2019). Besides, they are considered not only a source of nutrition but also health-promoting foods providing functional properties in terms of preventing food pathogens, anti-cancer, or antioxidants (Şanlier et al., 2019; Tasdemir & Sanlier, 2020; Abd-Ellatif et al., 2022; Kim et al., 2022;). The fermentation process is the most ancient and economical approach to producing food with high nutritional value and long-term preservative properties (Şanlier et al., 2019). Lactic acid bacteria (LAB) have been currently considered the most studied strain in food fermentation (Saguibo et al., 2019). These microbes are popularly characterized by their property to produce lactic acid as a main product of their anaerobic metabolism and significantly affect nutritional and sensorial properties of fermented foods (Ruiz-Rodriguez et al., 2019). Besides, the fermentation products from LAB, such as exopolysaccharides can be alternatives to food additives as emulsifiers, thickening agents, stabilizers, or texturizers (Korcz & Varga, 2021).

LAB, which are gram-positive strains and facultative anaerobic microorganisms, usually convert carbohydrate substrates into organic acids to create fermented foods with instinct flavors such as cheese, yogurt, sourdough, and sauerkraut (Lamont et al., 2017). Besides, the use of LAB also offers a great deal of health benefits as they potentially produce bioactive compounds (Garsa et al., 2014).

LAB, commonly *Lactobacillus*, have been mostly found in the human intestine with numerous health-promoting benefits due to their probiotic properties (Lim et al., 2018). The consumption of LAB-fermented foods was reported to positively control body weight (Mozaffarian et al., 2011). LAB fermented milk was found to reduce muscle pain (Iwasa et al., 2013) while kimchi, a traditional Korean product, was noted with anti-obesity and anti-diabetic properties (An et al., 2013). The antibacterial activity of LAB is also a crucial property that contributes to the shelf-life of fermented products. *L. plantarum*, *L. acidophilus*, *L. casei*, and *L. reuteri* were reported to exhibit the growth of food pathogens *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in ready-to-eat vacuum-packaged meat products (Awaisheh & Ibrahim, 2009). *L. plantarum* strain C11 was demonstrated to inhibit the growth of *Streptococcus agalactiae* strains by producing bacteriocins (Bodaszewska-Lubas et al., 2012). *L. paracasei* strain MRS-4 was reported to show very strong antibacterial activity against *Alicyclobacillus acidoterrestris* in a study done by Ju et al. (2021).

LAB can be isolated from different sources such as milk, soil samples, fresh water, poultry farm, fermented vegetables, fermented meats, or fermented cereals (Barakat et al., 2011; Oguntoyinbo & Narbad, 2015; Salmerón et al., 2015). They can also be found in raw materials from flowers, fruits, and vegetables (Naeem et al., 2012). The microbial population of vegetables and fruits ranges from 10^5 to 10^7 CFU/g, in which LAB only contribute to a small segment of microbiota, around 10^2-10^4 CFU/g (Di Cagno et al., 2013). It should be noted that different strains of LAB in fruits or vegetables can produce fermented products with distinct flavors and different profiles of bioactive compounds as well as their functional properties (Liu et al., 2018). To date, the isolation and characterization of LAB strains from fruits and vegetables are rarely discussed. The exploration of new LAB strains could be a potent candidate in the food industry. Guava (*Psidium guajava* L.) is one of the most commercially important fruits in many tropical/subtropical countries (Singh, 2011). Viet Nam has been considered one of the largest guava fruit exporters and guava fruits are also favorably freshly consumed (Le et al., 2021). In this study, we aimed to isolate some LAB strains with high lactic acid production and strong antibacterial activity from guava fruit in Viet Nam. The expected result is expected to characterize the new strain of LAB to contribute to the development of fermented products in the food industry.

2. MATERIALS AND METHOD

2.1. Materials

Guava fruits were harvested in different regions, including Tam Nong commune and Hoa Long commune (Dong Thap province), Dong Phuoc commune (Can Tho city). *Escherichia coli* was supplied from the Fermentation Technology Laboratory, Institute of Food and Biotechnology, Can Tho University.

Medium: De Man, Rogosa and Sharpe (MRS) (HiMedia, India), Ong Tho sweetened condensed milk (Vinamilk, Viet Nam), unsweetened fresh milk (Vinamilk, Viet Nam), and some chemicals were provided from commercial standard supplies.

2.2. Isolation of LAB from guava fruit

The prepared guava sample (20 g) was added to the sterile MRS broth (200 mL) in the 250 mL flask. The flask was then incubated at 30°C for 48 h. The inoculum (1 mL) was prepared by 10-fold serial dilution with sterile water in a test tube. An aliquot (100 µL) at each concentration was spread-plated on the MRS agar medium, then incubated for 48 h at 37°C. After 48 h of incubation for visible growth, each different isolate was collected and sub-cultured in for three times to achieve better purity of each isolate (Trindade et al., 2022).

2.3. Morphological and biochemical analysis of isolates

The colony morphology was observed under a stereoscope with color, form, edge, elevation, and diameter. The cell morphology was observed under a microscope (Olympus DP12 BX41, Olympus Corporation, Tokyo, Japan) with 1000x magnification.

The biochemical characteristics of the bacteria were determined by Gram staining, catalase (Ong et al., 2012), oxidase (Shields & Cathcart, 2010), indole, and lactic acid production (Xiao et al., 2015).

2.4. Evaluation of lactic acid production capacity

Each culture of LAB (1 mL) was added to 9 mL of MRS broth and incubated at 37°C, and the lactic acid content was determined after 72 h of culture by titration with 0.1 M NaOH and using phenolphthalein as a color indicator.

2.5. Evaluation of *E. coli* inhibitory ability

The antibacterial test was conducted by the well-diffusion agar method against *E. coli.* The *E. coli* suspension (50 µL) with a density of 10^6 CFU/mL was prepared on the NA plate. On the plate, five wells were made with a diameter of 4 mm (Mummed et al., 2018). The suspension of LAB in MRS broth at a density of 108 CFU/mL (50 µL) was poured into the wells of the NA plate. Ampicillin (0.1 mg/mL) and sterile water served as positive and negative controls, respectively. Then, the plate was incubated at 37°C for 24 h before measuring the diameter of the inhibition zone (mm). The diameter of the antibacterial zone (DK) is calculated by the formula:

$$DK\ (mm) = D1 - D0$$

In which: D1 is the total diameter (mm), and D0 is the diameter of the agar well (4 mm).

The antibacterial level of LAB strains is determined according to the convention of Leska et al. (2020). No antibacterial properties; Weak antibacterial properties: 1 mm $<DK<$ 6 mm; Medium antibacterial properties: 6 mm $\leq$ DK$<$ 11 mm; Strong antibacterial properties: 11 mm $\leq$ DK$\leq$ 16 mm; Very strong antibacterial properties: DK $\geq$ 16

2.6. Identification of LAB

The DNA was extracted according to Ong et al. (2012). The amplification of the genomic 16S rRNA region was performed using two primers, 1492R (5' TACGGTTACCTTGTTACGACT-3') and 27F (5'-AGAGTTTGATCCTGGCTC-3'). The 16S rRNA gene segment was then sequenced using the Sanger sequencing method. Once the sequencing results were obtained, a BLAST analysis was conducted on the NCBI system to compare the sequences with those of similar published strains. Sequences from the BLAST results of two bacterial strains in the study were compared with the 16S rRNA gene database of bacteria and archaea available in the NCBI database. Multiple sequence alignment was performed using the Clustal W algorithm in BioEdit version 7.2.1.

2.7. Investigation of the effects of guava juice ratio, milk ratio, and bacterial density on the fermentation process and quality of yogurt products

Conduct yogurt fermentation supplemented with guava pear with the ratio of guava pear juice added is 2%, 5% and 8%; the ratio of unsweetened fresh milk and condensed milk is 1%, 2% and 4% and the density of LAB bacteria is arranged as 3, 5, and 7 logCFU/mL. The finished yogurt product fermentation temperature and fermentation time are 30ºC for 16 h. After incubation, pH, total soluble solids (°Brix), and lactic acid concentration (g/L) were measured. Sensory evaluation was also performed according to TCVN 7030:2002. Yogurt is evaluated according to the TCVN 7030:2002 standard, including three criteria: color, taste. Each criterion is evaluated from 1 to 5 points with the score: Excellent - 5 points; Very good - 4 points; Good - 3 points; Fair - 2 points; Poor - 1 point. The sensory results are the sum of the 3 criteria. The optimal formula is the suitable parameter for the yogurt fermentation process.

2.8. Statistical analysis

Each experimental data was collected and graphed on Excel 2013 software (Microsoft Corporation, USA). Minitab 16 software (Minitab, Inc., Pennsylvania, USA) was utilized to analyze ANOVA test and Turkey HSD was used to compare mean values at the significant level of 5%.

3. RESULTS AND DISCUSSION

3.1. Isolation of LAB strains

The study isolated 20 LAB strains from guava and described in Table 1 and Figure 1. The colonies of all isolates showed a milky white color, circular, smooth, convex, and the diameter size ranging from 1 to 2 mm. While the morphological appearance of each isolate under the microscope showed significant differences, four typical shapes were identified, including short rod, oval, cocci, and long rod, among the 20 LAB isolates. All LAB were Gram-positive, catalase-negative, oxidase-negative, capable of degrading $CaCO_3$, and unable to form indole from tryptophan (Table 2). Colony and cell morphology of LAB isolated from guava were similar to previous studies by Vos et al. (2011), Ong et al. (2012), Ismail et al. (2018), and Mulaw et al. (2019). The diversity of LAB can be dependent on vectors such as birds and insects (Garcia et al., 2016; Ruiz-Rodriguez et al., 2019).

Table 1. Colony morphology of each LAB isolate

No.	Strains	Colony					Cell shape
		Color	Form	Edge	Elevation	Diameter (mm)	
1	TN1	Milky white	Circular	Smooth	Convex	1.0	Short rod
2	TN2	Milky white	Circular	Smooth	Convex	1.1	Oval
3	TN3	Milky white	Circular	Smooth	Convex	1.5	Cocci
4	TN4	Milky white	Circular	Smooth	Convex	1.2	Short rod
5	TN5	Milky white	Circular	Smooth	Convex	1.2	Short rod
6	TN6	Milky white	Circular	Smooth	Convex	2.0	Cocci
7	TN7	Milky white	Circular	Smooth	Convex	1.0	Oval
8	LV1	Milky white	Circular	Smooth	Convex	1.5	Short rod
9	LV2	Milky white	Circular	Smooth	Convex	1.0	Short rod
10	LV3	Milky white	Circular	Smooth	Convex	1.5	Oval
11	LV4	Milky white	Circular	Smooth	Convex	1.5	Oval
12	LV5	Milky white	Circular	Smooth	Convex	1.1	Short rod
13	LV6	Milky white	Circular	Smooth	Convex	1.0	Short rod
14	LV7	Milky white	Circular	Smooth	Convex	1.0	Long rod
15	LV8	Milky white	Circular	Smooth	Convex	1.5	Cocci
16	LV9	Milky white	Circular	Smooth	Convex	2.0	Short rod
17	LV10	Milky white	Circular	Smooth	Convex	1.2	Short rod
18	CTA1	Milky white	Circular	Smooth	Convex	1.5	Short rod
19	CTA2	Milky white	Circular	Smooth	Convex	1.1	Cocci
20	CTA3	Milky white	Circular	Smooth	Convex	1.5	Cocci

Note: TN: isolates from Tam Nong commune, LV: isolates from Hoa Long commune, and CTA: isolates from Dong Phuoc commune. Colonies were described on the MRS medium after 24 h incubation.

Table 2. Morphological and biochemical activities of each LAB isolate

No.	Strains	Gram	Catalase	Oxidase	Indole	$CaCO_3$
1	TN1	+	-	-	-	+
2	TN2	+	-	-	-	+
3	TN3	+	-	-	-	+
4	TN4	+	-	-	-	+
5	TN5	+	-	-	-	+
6	TN6	+	-	-	-	+
7	TN7	+	-	-	-	+
8	LV1	+	-	-	-	+
9	LV2	+	-	-	-	+
10	LV3	+	-	-	-	+
11	LV4	+	-	-	-	+
12	LV5	+	-	-	-	+
13	LV6	+	-	-	-	+
14	LV7	+	-	-	-	+
15	LV8	+	-	-	-	+
16	LV9	+	-	-	-	+
17	LV10	+	-	-	-	+
18	CTA1	+	-	-	-	+
19	CTA2	+	-	-	-	+
20	CTA3	+	-	-	-	+

Note: Gram-positive or positive biochemical reactions are denoted by (+), while negative reactions are denoted by (-).

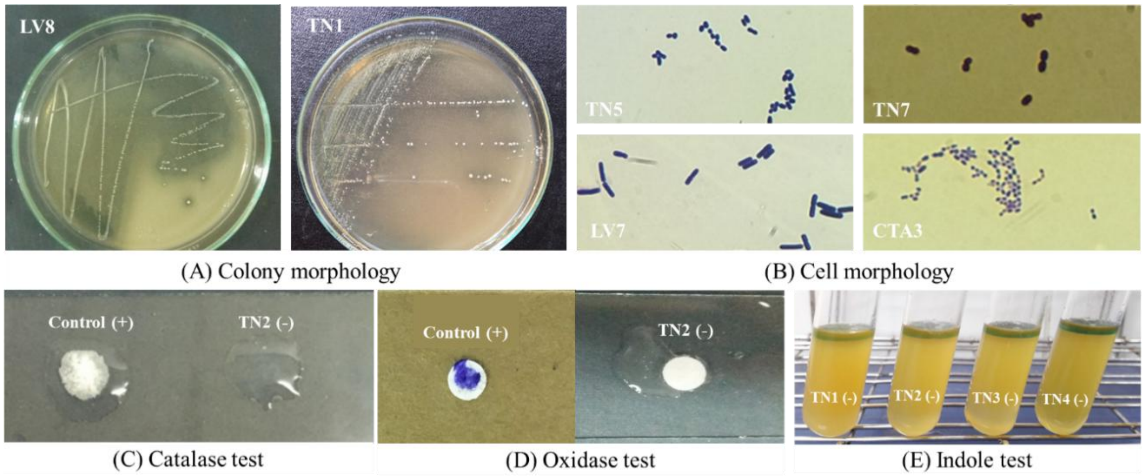

Figure 1. Morphology and biochemical characteristics of LAB bacteria

Note: (A) Colony morphology on MRS medium after 24 hours; (B) Cell morphology observed under a microscope at 1000X magnification; (C) Catalase test; (D) Oxidase test; (E) Indole test.

3.2. Total acid content produced by LAB isolates

All the LAB showed that capacity producing organic acids after 72 h of fermentation by decomposing $CaCO_3$. The total acid content produced from each LAB isolate is recorded in Figure 2. There were 5 LAB strains, including TN2, LV1, LV5, LV6, and LV10 showed a high acid production capacity. The highest acid content was produced by strain TN2 (8.7±0.14 g/L), while the lowest one was assigned to the strain CTA1 (3.63±0.05 g/L). The organic acid production capacity of LAB depends on the isolation source (Sheeladevi & Ramanathan, 2011; Ruiz-Rodriguez et al., 2019). Besides that, low pH tolerance affects organic acid production (Ayyash et al., 2018). Strain TN2 showed very high tolerance in a low pH environment and could produce organic acids. Moreover, organic acid processing can be influenced by the range of carbohydrate sources (Sifeeldein et al., 2019). *Lactobacillus* was classified into the homofermentative group because it mainly converts glucose into organic acids such as lactic acid, acetic acid, formic acid, and depends on their fermentability (Hofvendahl & Hahn–Hägerdal, 2000; Ruiz-Rodriguez et al., 2019). Furthermore, some other typical genera in this group are *Streptococcus* and *Pediococcus* (Sheeladevi & Ramanathan, 2011). In this study, the ability of isolated bacterial strains to ferment cow milk was investigated and demonstrated.

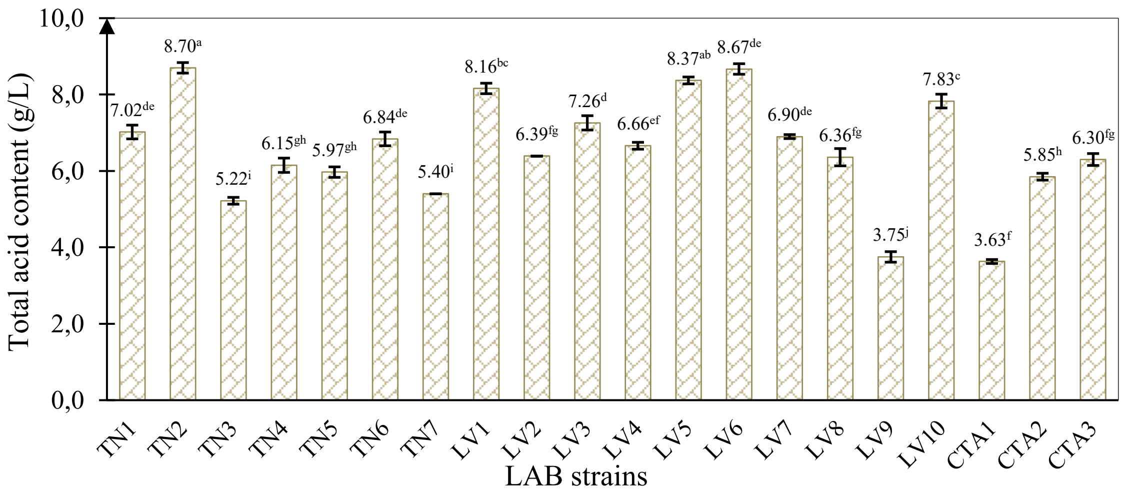

Figure 2. Total acid content produced by each LAB strain after 72 h

3.3. Antibacterial activity against *E. coli*

Table 3. Diameter of the inhibition zone of each LAB isolate against *E. coli*

No.	Strains	Diameter of halo (mm)	Antibacterial ability
1	TN2	$12.33^{b}\pm2.08$	+++
2	LV2	$8.00^{bc}\pm2.65$	++
3	LV4	$5.33^{c}\pm0.00$	+
4	LV5	$7.00^{c}\pm2.65$	++
5	LV9	$5.00^{c}\pm1.00$	+
6	LV10	$4.33^{c}\pm1.53$	+
7	Ampicillin	$27.26^{a}\pm0.06$	++++
8	Sterile water	4.00 ± 0.00	-

Note: Each treatment was replicated three times. Means ± SD followed by the same letter are not significantly different at the 5% level in the same column using Tukey's test. (+): weak antibacterial; (++): medium antibacterial; (+++): strong antibacterial; (++++): very strong antibacterial

Six out of 20 LAB isolates, including TN2, LV2, LV4, LV5, LV9, and LV10, were found to induce a bactericidal effect against *E. coli* (Table 3). Penicillin, as a positive control, showed very strong antibacterial activity, obtaining an inhibition zone diameter of 27.26 ± 0.57 mm. This was consistent with many reported studies using penicillin as a positive control (Ahmed et al., 2010; Koona & Budida, 2011). Six LAB strains showed an inhibition zone diameter ranging from 4.33 mm to 12.33 mm. Obviously, the strain TN2 showed the highest antimicrobial activity (12.33±2.08 mm). Lactobacillus isolates from Chinese fruits, flowers, and traditionally fermented Ethiopian foods had the ability to inhibit pathogenic bacteria (Sakandar et al. 2019; Mulaw et al. 2019). Microorganisms inhibited pathogens by nutritional competition, bacteriocin, and organic acid. *Lactobacillus* secretes bacteriocins and produces organic acids to reduce the pH of the environment to control pathogens (Barbosa et al., 2018; Trindade et al., 2022). A study by Epand et al. (2016) found that bacteriocins produced by lactic acid bacteria (LAB) are antibacterial peptides effective in inhibiting or destroying pathogenic bacteria in the intestine. These bacteriocins are naturally occurring proteins synthesized by ribosomes and subsequently degraded by proteolytic enzymes. As a result, pathogens are unable to develop resistance to these bacteriocins within the intestinal environment (Sánchez et al., 2008; Umu et al., 2017). In summary, as the strain TN2 was observed with the highest antibacterial activity and organic acid-producing capacity, this strain was selected to be identified by sequencing 16S rRNA.

3.4. Identification of LAB isolate

In this study, according to the antimicrobial test and organic acid-producing capacity results of 20 LAB strains. The strain TN2, which showed the highest antimicrobial activity and acid-producing capacity, was analyzed by sequencing of the 16S rRNA region. The gene sequences showed that TN2 had 99.31% similarity in sequence (Table 4) with *Lactococcus lactis* (the MN860000.1 sequence was registered in NCBI). This strain has been commonly used as a starter culture in fermented food products (Vos et al., 2011). Kuda et al. (2016) suggested that *L. lactis* could be a potent starter culture for high-quality milk and soymilk fermentation. Besides, its antimicrobial activity has been demonstrated in many previous studies. *L. lactis* Z11 isolated from an Arabian yogurt exhibited an inhibitory effect against some LAB strains and food pathogens such as *Listeria monocytogenes, Bacillus cereus,* and *Staphylococcus aureus* (Enan et al., 2013). Hwanhlem et al. (2013) indicated that *L. lactis* could eradicate *Listeria innocua, Staphylococcus carnosus, Brochothrix thermosphacta,* and *Clostridium botulinum* due to its bacteriocin secretion. The bacteriocin-producing capacity of *L. lactis* was observed to improve the food safety of the fermented products due to its effective bactericidal effect (Noonpakdee et al., 2003).

Table 4. The comparison of sequencing between TN2 and the data on NCBI

Strains	Length (nt)	Coverage	Identities	Species	Accessions
TN2	1446	99%	99.31%	*Lactococcus lactis* strain 4477	MT 545002.1
			99.24%	*Lactococcus lactis* strain YL-10	MW 007835.1
			99.17%	*Lactococcus lactis* strain sysl5	OL 823008.1

3.5. Effects of guava juice ratio, milk ratio, and bacterial density on fermentation process and quality of yogurt products

In general, the lactic acid content produced by LAB strains was greatly affected by bacterial density, guava juice ratio, and milk ratio. After 16 hours of fermentation, treatment 26 gave the highest lactic acid content of 9 g/L. The lactic acid content in this experiment was not statistically different from treatments 25 and 27, with lactic acid content of 8.4 g/L and 8.85 g/L, respectively. The above research results show that the higher the bacterial density, the greater the lactic acid content produced, leading to a decrease in pH value and a tendency to acidify (Table 5).

Table 5. Effect of density of bacteria, guava juice, and milk on fermentation

Treatment	Bacteria (log CFU/mL)	Guava juice (%)	Milk: co-milk	pH after	°Brix before	°Brix after	Lactic acid content (g/L)	Sensory evaluation (Score)
1	3	2	1	6.23^{b}	43	41.67	4.80^{l}	3.07^{l}
2	3	2	2	6.26^{c}	34	32.33	5.10^{kl}	3.07^{l}
3	3	2	4	6.21^{d}	29	23.67	5.25^{jkl}	3.13^{l}
4	3	5	1	6.43^{a}	43	42.00	5.10^{kl}	3.10^{l}
5	3	5	2	5.99^{g}	34	32.00	5.40^{ijkl}	3.13^{l}
6	3	5	4	6.17^{de}	29	22.67	5.55^{hijk}	3.07^{l}
7	3	8	1	6.28^{bc}	43	41.67	5.40^{ijkl}	3.20^{k}
8	3	8	2	6.17^{de}	34	32.00	6.00^{fghi}	3.13^{l}
9	3	8	4	6.14^{e}	29	23.33	5.85^{ghij}	3.40^{ik}
10	5	2	1	6.27^{c}	43	40.33	6.15^{efgh}	3.37^{k}
11	5	2	2	6.15^{e}	34	31.33	6.60^{ef}	3.53^{ijk}
12	5	2	4	6.09^{f}	29	22.00	6.45^{efg}	3.50^{ijk}
13	5	5	1	6.18^{de}	43	40.33	6.30^{efg}	3.50^{ijk}
14	5	5	2	5.97^{g}	34	31.00	6.60^{ef}	3.63^{i}
15	5	5	4	6.06^{f}	29	21.33	6.75^{e}	3.50^{ijk}
16	5	8	1	6.07^{f}	43	39.67	6.60^{ef}	3.63^{i}
17	5	8	2	6.00^{g}	34	30.33	6.75^{e}	3.60^{i}
18	5	8	4	5.98^{g}	29	21.33	6.60^{ef}	3.53^{ij}
19	7	2	1	5.23^{ij}	43	38.67	7.80^{cd}	7.03^{h}
20	7	2	2	5.21^{ijk}	34	28.33	8.10^{cd}	7.40^{f}
21	7	2	4	5.21^{ijk}	29	22.67	7.80^{cd}	7.43^{ef}
22	7	5	1	5.24^{i}	43	38.00	7.50^{d}	7.23^{g}
23	7	5	2	5.20^{ijk}	34	27.67	8.10^{cd}	7.83^{c}
24	7	5	4	5.19^{jkl}	29	21.33	8.25^{bc}	7.57^{de}
25	7	8	1	5.30^{h}	43	37.67	8.40^{abc}	7.70^{cd}
26	7	8	2	5.14^{l}	34	26.33	9.00^{a}	8.40^{a}
27	7	8	4	5.17^{kl}	29	21.33	8.85^{ab}	8.17^{b}

Note: Each treatment was replicated three times. Within each column, mean values followed by the same letters represent statistically insignificant differences at the 5% significance level according to Tukey's test.

The results showed that the highest lactic acid content is 9 g/L and the highest sensory evaluation scores according to TCVN 7030:2002 at treatment 26 with a bacterial density of 7 log CFU/mL, 8% guava juice, and a milk supplement ratio of 2. This result was chosen as the appropriate parameter for the yogurt fermentation process.

After 16 hours of incubation, the pH, Brix, and total acid content of the treatments were recorded and are presented in Table 5. In the milk environment, LAB strains converted lactose and sucrose into lactic acid. Lactose was hydrolyzed into two monosaccharides, glucose and galactose, while sucrose was converted into glucose and fructose. The LAB metabolize these sugars through the Embden-Meyerhof-Parnas (EMP) pathway, resulting in the production of pyruvic acid, which is then converted into lactic acid. The density of the bacterial culture significantly affects the observed values; a higher cell count enhances fermentation efficiency and increases lactic acid production. The lactic acid concentration at a cell density of log 7 is notably higher, ranging from 7.8 to 9.00 g/L, compared to log 5, which has a concentration between 6.15 and 6.75 g/L, and log 3, with lactic acid levels ranging from 4.8 to 6.00 g/L. Additionally, the pH values gradually decrease with increasing bacterial density, with log 7 exhibiting the lowest pH due to the higher lactic acid production. Moreover, the Brix value also significantly decreases in treatments with a cell density of log 7. The ratio of fresh milk to condensed milk also influences the fermentation process. A 1:1 ratio shows that a higher sugar concentration inhibits fermentation, resulting in slower rates compared to ratios of 2.5 and 4, leading to a higher pH value in the 1:1 milk ratio compared to the 4 and 2.5 ratios. The lactic acid content in treatments with the 1:1 ratio is also lower and significantly different from the other treatments. Furthermore, the percentage of added guava impacts the fermentation process. Guava contains essential minerals and vitamins, particularly vitamin C, which, along with lactic acid bacteria, promotes fermentation. Therefore, the treatment with 8% guava exhibits a higher lactic acid content and shows significant statistical differences compared to the other treatments.

4. CONCLUSION

In this study, 20 LAB strains from guava fruits were successfully isolated. These strains showed typical morphological and biochemical properties of lactic acid bacteria. All LAB isolates exhibited organic acid-producing capacity, with TN2 being the strongest. Whereas 6 out of 20 strains were found to induce bactericidal effects against *E. coli*. TN2 was also considered the strain with the highest antibacterial activity. For application to ferment yogurt supplemented with 8% guava fruit juice, the ratio of fresh milk and condensed milk is 2, bacterial density 7 logCFU/mL at pH 6.0, giving the highest organic acid producing capacity and the best sensory evaluation. The strain TN2 was identified as *Lactococcus lactis,* which is well known as a strong starter culture in the fermentation process. The strain TN2 can be a potent starter culture to develop fermented food products, as well as contribute to extending the shelf-life of the products due to its high antibacterial activity.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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