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FOOD TECHNOLOGY

Isolation and selection of γ-aminobutyric acid producing lactic acid bacteria and application in GABA-enriched tomato juice fermentation

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ABSTRACT: Tomatoes (*Solanum lycopersicum* L.) are nutrient-rich fruits with a high glutamic acid content, making them a suitable source for producing γ-aminobutyric acid (GABA) through the action of lactic acid bacteria (LAB). LAB plays a pivotal role in lactic fermentation, enhancing the taste and nutritional value of food products, often contributing to their health benefits. This study isolated GABA-producing LAB from tomatoes and assessed their potential for producing GABA-enriched fermented tomato juice. The results indicated the isolation of 15 LAB strains from the samples of five different tomatoes. All of these strains demonstrated the capability to produce GABA, with levels ranging from 1.732 to 3.113 mg/mL, as determined using thin-layer chromatography (TLC). Through sequencing, the promising strain TO42, identified as *Weissella cibaria*, was selected for tomato juice fermentation. Furthermore, GABA-enriched tomato juice was successfully fermented at 15 °Brix for 72 h, resulting in the highest recorded GABA and lactic acid content of 9.185 ± 0.398 mg/mL and 13.05 ± 1.56 g/L, respectively.
Key words: Lactic acid fermentation, tomato juice, thin layer chromatography, γ-aminobutyric acid (GABA).

Isolamento e seleção de bactérias lácticas produtoras de ácido γ-aminobutírico e aplicação na fermentação de suco de tomate enriquecido com GABA

RESUMO: O tomate (*Solanum lycopersicum* L.) é uma fruta rica em nutrientes e com alto teor de ácido glutâmico, tornando-o uma fonte adequada para a produção de ácido γ-aminobutírico (GABA) através da ação de bactérias ácido lácticas (BAL). As BAL desempenham um papel fundamental na fermentação láctica, melhorando o sabor e o valor nutricional dos produtos alimentares, contribuindo frequentemente para os seus benefícios para a saúde. Este estudo teve como objetivo isolar BAL produtoras de GABA de tomates e avaliar seu potencial para a produção de suco de tomate fermentado enriquecido com GABA. Os resultados indicam o isolamento de 15 cepas de BAL a partir de amostras de cinco tomates diferentes. Todas essas cepas demonstraram capacidade de produzir GABA, com níveis variando de 1,732 a 3,113 mg/mL, conforme determinado por cromatografia em camada delgada (CCD). Através de sequenciamento, a cepa promissora TO42, identificada como *Weissella cibaria*, foi selecionada para fermentação de suco de tomate. Além disso, o suco de tomate enriquecido com GABA foi fermentado com sucesso a 15 °Brix por 72 h, resultando no maior teor registrado de GABA e ácido láctico de 9.185 ± 0.398 mg/mL e 13.05 ± 1.56 g/L, respectivamente.
Palavras-chave: fermentação láctica, suco de tomate, cromatografia em camada delgada, ácido γ-aminobutírico (GABA).

INTRODUCTION

In recent years, there has been significant interest in research on functional foods and healthy products containing beneficial bioactive compounds. Fermented fruit juice, a beverage produced through the lactic acid fermentation process, is known for its ability to preserve and enhance the nutritional properties of the juice. Non-dairy functional beverages have substantial commercial potential because of their capacity to provide appropriate probiotics, vitamins, total phenols, amino acids, and exopolysaccharides. Specifically, their sensory properties, antioxidant activities, anti-cancer, anti-diabetic, and anti-inflammatory effects have been studied extensively. All of these advantages are closely linked to the use of LAB strains and plant-based materials such as fruits and vegetables (SZUTOWSKA, 2020).

LAB plays a crucial role in lactic fermentation because of its ability to convert sugars into lactic acid, which reduces the pH of the food and inhibits the growth of pathogens. In addition, LAB is known to increase the nutritional value of fermented foods through their growth and development. Secondary metabolites with high biological value are produced during their growth, such as aroma compounds, organic acids, phenolics, bacteriocins, vitamins, and exopolysaccharides. Notably, they also produce γ-aminobutyric acid (GABA) (WANG et al., 2021; PLESSAS, 2021). GABA is a non-protein

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amino acid that acts as a neurotransmitter in the nervous system by blocking or preventing specific brain signals and reducing nervous system activity. It produces a calming effect that helps reduce feelings of anxiety, stress, and fear. Consequently, GABA is used as an adjuvant to ameliorate insomnia and depression, boost immunity, manage menopausal syndrome, regulate blood pressure, combat obesity, and improve visual function (CUI et al., 2020). Some LAB strains can produce GABA through the enzyme L-glutamate decarboxylase (GAD, EC 4.1.1.15), which increases the resistance of LAB to acidic environments by catalyzing the decarboxylation of L-glutamate to GABA and carbon dioxide.

Therefore, foods with a high L-glutamate content, such as tomatoes (*Solanum lycopersicum* L.), are suitable for the production of GABA-enriched fermented juice using LAB promoter strains. In addition, tomatoes are rich in essential nutrients such as β-carotene, lycopene, vitamins A, and vitamin C, which are substances that help the body fight against oxidation (PAOLO et al., 2018). This study isolated LAB from tomatoes with GABA-producing ability and investigate their potential for the production of GABA-enriched tomato juice.

MATERIALS AND METHODS

Materials

Five tomato samples from various sources were purchased from a local market in Can Tho City, Vietnam. MRS (De Man, Rogosa, and Sharpe; Merck, Germany) medium was used for LAB isolation. The chromatography paper used in this study was TLC Silica gel 60 F_{254} (Merck, Germany). Other chemicals, such as GABA (Merck, Germany), ninhydrin (Xilong, China), monosodium glutamate (MSG, Ajinomoto, Vietnam), $CuSO_4.5H_2O$, $CaCO_3$, NaOH, HCl, H_2O_2, $NaHSO_3$, pectinase, crystal violet, iodine, 1% phenolphthalein, ethanol, distilled water, and agar, were purchased from standard commercial suppliers.

Methods

Isolation of LAB from tomatoes

To initiate the experiment, 10 mL of tomato juice was cultured in 90 mL of MRS broth at 37 °C for 48 h, and then 1 mL of it was mixed with 9 mL of 0.85% (w/v) NaCl. Subsequently, 0.1 mL of the solution at 10^{-4} and 10^{-5} concentration levels were spread onto MRS agar plates containing 0.5% (w/v) $CaCO_3$ and anaerobically incubated at 37 °C for 48 h (KHUNAJAKR et al., 2008). Colonies displaying $CaCO_3$ lysis zones were selected and restreaked to obtain pure strains. These strains were characterized using Gram staining, catalase, oxidase, and motility assays.

Determination of GABA-producing LAB strains using Thin Layer Chromatography (TLC)

Each LAB strain was incubated in 20 mL of MRS medium at 37 °C for 36 h until the cell density reached approximately 10^9 CFU/mL. Then, 1 mL of this medium was transferred to a test tube containing 9 mL of MRS medium supplemented with 3% MSG (w/v) and an initial pH of 4.5. Static fermentation was carried out at 37 °C for 48 h to assess the GABA-producing ability of LAB using the improved thin-layer chromatography (TLC) method by LI et al. (2009).

Following 48 h of fermentation, 1 mL of the culture was aspirated into an Eppendorf tube and centrifuged at 10.000 rpm for 10 min to remove the biomass. Subsequently, 2 µL of the supernatant was dotted onto an aluminum sheet coated with silica gel 60 F_{254} (5×10 cm), which had been activated by drying at 90 °C for 30 min. Each dot was spaced 1 cm apart and 1.5 cm from the solvent, and after drying for 3, the TLC plate was placed in a chamber containing 20 mL of solvent composed of n-butanol, acetic acid, and water (5:3:2) supplemented with 1.2% ninhydrin (w/v). The TLC plate was removed when the chromatographic solvent reached 0.5 cm from the top and dried at 70 °C for 80 min to visualize the GABA spots. The determination of the GABA-producing ability of LAB strains was based on the retardation factor (R_f) of the fermentation broth compared with that of the GABA standard solution (2 mg/mL). The purified GABA spots on the chromatographic plates were cut and extracted with 5 mL of a 75% ethanol solution containing 0.6% $CuSO_4.5H_2O$ (w/v) in a 38:2 (v/v) ratio. The mixture was placed on a shaker until the pure GABA spot completely dissolved in the solvent. The absorbance was measured at 512 nm to determine the GABA content in the fermentation broth produced by LAB. GABA quantification in the samples was performed based on the GABA standard curve (0.5-5 mg/mL) using the same method.

Investigating the lactic acid fermentation ability of GABA-producing LAB strains

GABA-producing LAB strains were propagated in 20 mL of MRS medium at 37 °C for 36 h until the cell density reached approximately 10^9 CFU/mL. Then, 1 mL of this culture was transferred to conical flasks containing 99 mL of sterilized MRS medium adjusted to pH 6.5. The mixture

was anaerobically incubated at 37 °C and statically fermented for 120 h.

The lactic acid content produced during the 120 h fermentation was determined using the adjusted Therner titration method (NGUYEN & HWANG, 2016). Specifically, 1 mL of the fermentation solution was placed in a test tube and supplemented with 1-2 drops of 1% phenolphthalein. The titration was performed by adding 25 µL of 0.1 N NaOH at each step and counting the number of additions (X) until the solution turned a stable pink color for 30 seconds. The total acid content was calculated using the following formula: Lactic acid (g/L) = 0.009 × X × 0.025 × 1,000 where 0.009 is the conversion factor of total acid to lactic acid, corresponding to 1 mL of NaOH 0.1 N.

Identification of the selected GABA-producing LAB

DNA extraction of selected lactic acid bacteria was amplified using the 16S rRNA gene fragment with the primer pair 1492R (5'-TACGGTTACCTTGTTACGACT-3') and 27F (5'-AGAGTTTGATCCTGGCTC-3') (WEISBURG et al., 1991). The resulting PCR product was purified and sequenced using an automated sequence analyzer. The sequencing results were compared with the GeneBank of the NCBI database (www.ncbi.nlm.nih.gov/) using the BLAST tool to determine the species of the selected lactic bacteria strain.

Effect of the dissolved solid concentration on tomato juice fermentation

The experiment investigated the influence of the dissolved solid content (5, 10, 15, and 20 ºBrix) on the fermentation of GABA-enriched tomato juice by the selected bacteria. The LAB strain was cultured in 20 mL of MRS medium at 37 °C for 36 h until the cell density reached 10^9 CFU/mL.

In addition, ripe red tomatoes were selected as the main ingredient, washed, and allowed to dry naturally. The seeds were then removed, and tomato juice was extracted. The fermentation process for GABA-enriched tomato juice was conducted as follows: The fruit juice was supplemented with pectinase (50 mg/L) at room temperature (between 28-32 ℃) to enhance the recovery efficiency. Sucrose was added to adjust the Brix level to 5, 10, 15, and 20 ºBrix, while the initial pH was set to 4.5 using citric acid and Na_2CO_3. Subsequently, the tomato juice was pasteurized with $NaHSO_3$ (140 mg/L) for 2 h at room temperature (28-32 ℃). Finally, 100 mL of tomato juice was transferred into a flask and inoculated with 1% (v/v) lactic acid bacteria (10^9 CFU/mL), fermented under anaerobic conditions at 37 ℃.

Statistical analysis

All experiments were conducted in triplicate, and the data were processed and graphed using Microsoft Excel 2016 software (Microsoft Corporation, USA). The presented results are expressed as the mean ± standard deviation. Analysis of variance was performed using Rstudio software (Posit Software, USA), and Duncan's test was employed to compare mean values at a significant level of 5%.

RESULTS AND DISCUSSION

Isolation of LAB from tomatoes

Eighteen bacterial strains were isolated from tomato samples, of which 15 strains were identified based on their morphological, physiological, and biochemical characteristics. These strains exhibited typical features of the genus LAB: white colonies (clear white, ivory-white, milky white), spherical (cocci) or rod-shaped (bacilli) cells (Figure 1), Gram-positive, negative for catalase and oxidase, capable of degrading $CaCO_3$, and non-motile (Table 1). The observation results of bacterial colonies of various strains after 48 h of cultivation on MRS agar medium, shown in table 1, indicate that 18 bacterial strains are similar in colony morphology. All strains have round colonies, with smooth and convex surfaces and entire margins, except for strain TO23, which has a fimbriate margin. The color of the colonies of the 18 bacterial strains falls into three categories: 8 strains are milky white (44.44%), 6 strains are ivory white (33.33%), and 4 strains are clear white (22.22%). The size of individual colonies on the medium was recorded to vary from 1.0 to 4.0 mm.

In a study by SAJUR et al. (2007), 35 LAB strains were isolated from various tomato sauce samples using MRS-P agar medium (MRS medium supplemented with 1.3 µg/mL of pimaricin). All these strains were Gram-positive, catalase-negative, non-sporulating, and non-motile. The low pH (around 4.0-4.5) and the presence of organic acid molecules in tomatoes favor acid-tolerant microorganisms such as fungi and lactic acid bacteria. Additionally, WU et al. (2014) isolated 108 lactic bacteria strains from tomato residue, identifying them as *Lactobacillus harbinensis, L. manihotivorans, L. helveticus, L. camelliae, L. pontis, L. amylovorus, L. hilgardii, L. panis, L. vaginalis*, and *L. rapi.*

Determination of GABA-producing LAB strains by TLC

The chromatography results revealed that 15 isolated LAB strains from tomatoes were capable of

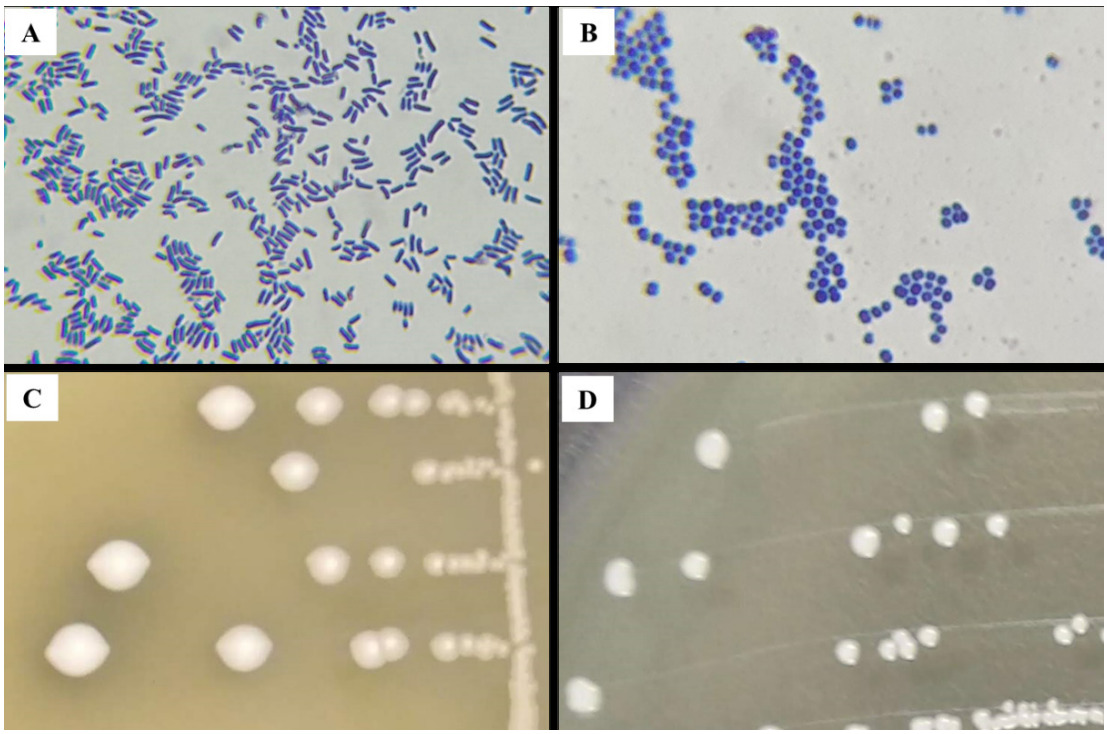

Figure 1 - Cell and colony morphology of TO42 (A, C) and TO13 (B, D) strains.

producing GABA in MRS medium supplemented with 3% (w/v) MSG after 48 h of fermentation at 37 °C. The GABA spots from all samples on the chromatography plate had an R_f of 0.3, similar to the standard GABA spots (2 mg/mL), as shown in figure 2.

LAB synthesizes GABA from L-glutamate through GAD, a process that is dependent on various factors such as pH, glutamate concentration, temperature, time, and culture medium (YOGESWARA et al., 2020). To enhance GABA production by LAB, MSG can be used as an additional glutamate source in liquid MRS medium to replace L-glutamine because of its high glutamate content (approximately 78% w/w).

The determined GABA-producing strains will be subjected to TLC chromatography with three replicates. The identified GABA spots on the TLC sheet were extracted with 75% ethanol solution and $CuSO_4.5H_2O$ 0.6% (w/v). The GABA content was calculated based on the GABA standard curve equation (0.5-5 mg/mL): $y = 0.0314x - 0.0074$, $R^2 = 0.9983$. The GABA content produced during the fermentation of 15 LAB strains ranges from 1.732 to 3.113 mg/mL (Figure 3). Among them, strain TO12 recorded the highest GABA content of 3.113 mg/mL, which was especially statistically significant ($P < 0.05$) with the remaining strains. Strains TO15, TO23, TO24, TO31, TO41, TO42, TO43, TO51, and TO52 have GABA contents in the range of 2.019-2.359 mg/mL, and there is almost no statistically significant difference between them. In particular, strains TO14, TO16, TO34, TO35, and TO44 showed lower GABA levels (1.732-1.796 mg/mL) and were different from the other two groups, in which TO35 had the lowest GABA level (1.732 mg/mL).

In the study by NAKATANI et al. (2022), the diversity of GABA-producing LAB strains from various types of Japanese pickles was demonstrated. After isolation and screening, 74 GABA-producing LAB strains were identified. These strains were determined to be *L. sakei* (24 strains), *L. plantarum* (23 strains), and *L. brevis* (27 strains). Among them, the *L. plantarum* KB1253 strain had the highest GABA production capability under optimal conditions, producing 25.4 ± 0.4 mg/mL GABA in the MRS medium. The study also revealed that *L. plantarum* KB1253 has two GAD genes (*gad*B1 and *gad*B2) that regulate GABA production. A typical

Table 1 - Morphological, physiological and biochemical characteristics of 18 bacterial strains isolated from tomatoes.

Strain	----------Colony----------	Cell shape	Gram test	Oxidase	Catalase	Motile
TO12	Circle, clear white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO13	Circle, ivory-white, smooth, convex, entired margin	Cocci (spherical)	+	-	+	No
TO14	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO15	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO16	Circle, ivory-white, smooth, convex, entired margin	Bacilli (long rod)	+	-	-	No
TO18	Circle, clear white, smooth, convex, entired margin	Bacilli (long rod)	+	-	+	No
TO22	Circle, ivory-white, smooth, convex, entired margin	Cocci (spherical)	+	-	+	No
TO23	Circle, clear white, smooth, convex, fringed margin	Bacilli (long rod)	+	-	-	No
TO24	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO31	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO34	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO35	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO41	Circle, milky white, smooth, convex, entired margin	Cocci (spherical)	+	-	-	No
TO42	Circle, ivory-white, smooth, convex, entired margin	Bacilli (long rod)	+	-	-	No
TO43	Circle, ivory-white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO44	Circle, milky white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO51	Circle, ivory-white, smooth, convex, entired margin	Bacilli (short rod)	+	-	-	No
TO52	Circle, clear white, smooth, convex, entired margin	Bacilli (long rod)	+	-	-	No

feature of LAB is its ability to form lactic acid during the fermentation of carbohydrates and to survive in an acidic environment mainly containing lactic acid, LAB has developed different antacid systems. In which, the ability of LAB to be acid resistant through the synthesis of GABA by decarboxylating the amino acid L-glutamine and consuming a proton (H^+) significantly contributes to maintaining the pH homeostasis of the et cytoplasm. According to CUI et al. (2020), foods rich in glutamate are an important source for isolating GABA-producing LAB. Lactic acid bacterial strains reported to have the ability to produce GABA include *Lactobacillus buchneri*, *Lactobacillus brevis*, *Lactobacillus paracasei*, *Lactiplantibacillus plantarum*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Therefore, GABA production among LAB strains also differs (YOGESWARA et al., 2020).

Lactic acid fermentation ability of GABA-producing LAB strains

The results in table 2 demonstrate that all 15 LAB strains were capable of producing lactic acid in the culture medium after 24 h of fermentation. Generally, the lactic acid content increased rapidly within the first 48 h of fermentation and then stabilized. This is because, during LAB growth, lactic acid is produced and accumulates in the culture medium, lowering the pH. When the pH becomes too low, it inhibits LAB activity. After 24 h of fermentation, the recorded lactic acid levels ranged from 7.05 to 19.20 g/L. Strains TO51 and TO52 adapted to the environment quicker than others, with the highest recorded lactic acid contents of 19.05 and 19.20 g/L, respectively. However, their lactic acid contents gradually decreased afterward. Strain TO42 exhibited the highest lactic acid content after 48 h of fermentation and remained stable for the following 3 days. Because of its stability and strong lactic acid fermentation activity, strain TO42 was selected for sequencing and testing in tomato juice fermentation.

In the research of PHONG et al. (2017) about the fermentation ability of *Lactobacillus* sp. Y1, the lactic acid content in the medium increased rapidly from the first 24-36 h of fermentation and began to decrease when the fermentation time lasted 48 h. KIM et al. (2009) similarly showed that GABA-producing LAB like *L. paracasei* reduced the fermentation medium pH from 6.5 to about 4.5 within 50 h of fermentation, which explains the lack of significant increase in lactic acid content quickly after 48 h of fermentation because the accumulation of lactic acid leads to decrease pH environmental, resulting in growth inhibition of LAB strains.

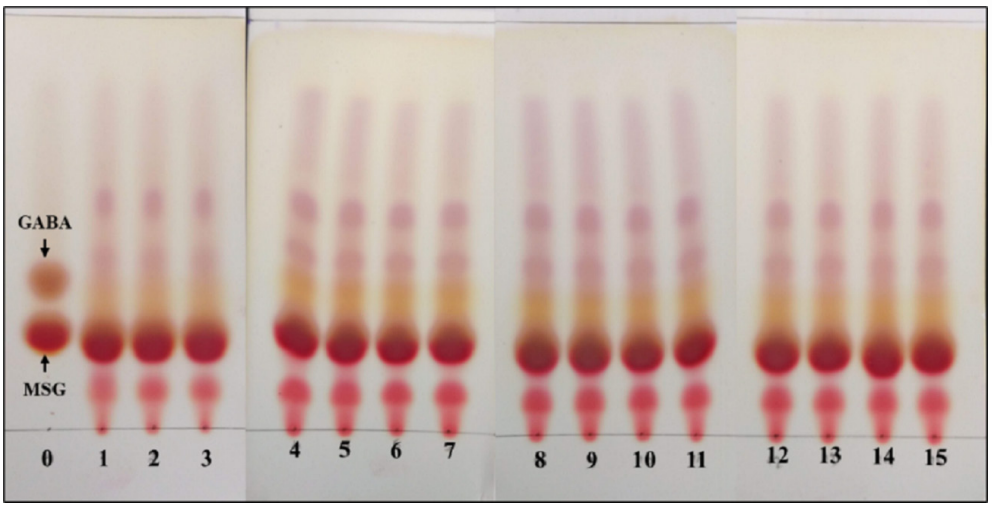

Figure 2 - GABA-producing of isolated LAB screening by TLC. *Lane 0* Mix GABA (2 mg/mL) and MSG (5 mg/mL) solution, *lane 1* strain TO12, *lane 2* strain TO14, *lane 3* strain TO15, *lane 4* strain TO16, *lane 5* strain TO23, *lane 6* strain TO24, *lane 7* strain TO31, *lane 8* strain TO34, *lane 9* strain TO35, *lane 10* strain TO41, *lane 11* strain TO42, *lane 12* strain TO43, *lane 13* strain TO44, *lane 14* strain TO51, *lane 15* strain TO52.

Identification of the selected GABA-producing LAB

The 16S rRNA sequencing result of the TO42 lactic acid bacterial strain yielded the sequence with a length of 939 bases. It was found that strain TO42 has a sequence highly similar to that of *Weissella cibaria* belongs to the lactic acid bacteria group, with a coverage of 98% and a similarity of 99.78% to the 16S rRNA sequence of *Weissella cibaria* 4370 (MT544906.1). The genus *Weissella* is divided into 16 strains that share many similar

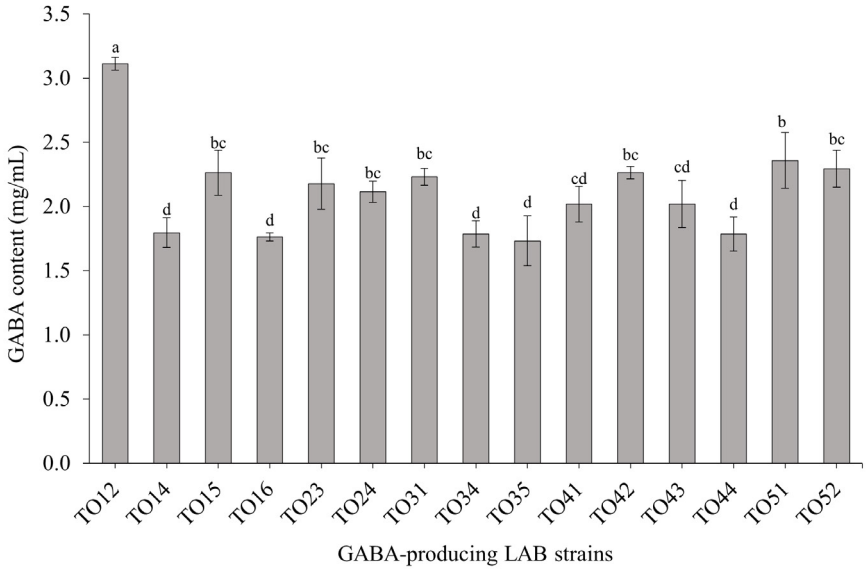

Figure 3 - GABA-producing content of LAB isolated from tomatoes.

Note: The results presented are the mean of 3 replicates and the standard deviation. Letters in the same columns represent statistically insignificant differences at the 95% confidence level ($P < 0.05$).

Table 2 - Titration results to determine lactic acid content in 120 h fermentation of 15 strains of GABA-producing lactic bacteria.

Strain	----------Lactic acid content (g/L)----------				
	24 h	48 h	72 h	96 h	120 h
TO12	8.18 ± 0.13^{h}	9.75 ± 1.13^{g}	11.1 ± 0.26^{h}	11.10 ± 0.52^{f}	10.80 ± 0.00^{f}
TO14	8.55 ± 0.00^{h}	9.60 ± 1.58^{g}	11.4 ± 0.52^{gh}	11.10 ± 0.52^{f}	10.80 ± 0.00^{f}
TO15	12.38 ± 0.23^{f}	$13.9\ 5\pm 1.19^{b}$	13.35 ± 0.26^{de}	13.50 ± 0.00^{c}	13.50 ± 0.00^{c}
TO16	13.50 ± 0.45^{cd}	12.00 ± 0.26^{d}	14.55 ± 0.26^{b}	15.00 ± 0.52^{b}	15.30 ± 0.00^{b}
TO23	13.20 ± 0.52^{de}	16.05 ± 0.52^{a}	14.70 ± 0.52^{b}	14.70 ± 0.52^{b}	15.00 ± 0.52^{b}
TO24	13.05 ± 0.78^{def}	10.35 ± 0.00^{efg}	13.50 ± 0.78^{cd}	13.50 ± 0.00^{c}	14.10 ± 0.52^{c}
TO31	13.95 ± 0.45^{bc}	13.65 ± 1.82^{bc}	14.25 ± 0.94^{bc}	14.40 ± 0.00^{b}	15.30 ± 0.00^{b}
TO34	14.40 ± 0.00^{b}	12.45 ± 0.52^{cd}	14.40 ± 0.00^{b}	15.00 ± 0.52^{b}	15.00 ± 0.52^{b}
TO35	8.40 ± 0.26^{h}	9.90 ± 0.45^{g}	10.50 ± 0.26^{h}	11.10 ± 0.52^{f}	11.10 ± 0.52^{ef}
TO41	12.68 ± 0.13^{ef}	11.70 ± 0.00^{de}	12.30 ± 0.52^{f}	11.70 ± 0.00^{e}	12.38 ± 0.98^{d}
TO42	7.05 ± 0.26^{i}	16.20 ± 0.00^{a}	18.30 ± 0.52^{a}	18.00 ± 0.00^{a}	18.60 ± 0.26^{a}
TO43	11.70 ± 0.00^{g}	10.20 ± 0.52^{fg}	10.80 ± 0.00^{h}	10.80 ± 0.00^{f}	11.85 ± 0.26^{de}
TO44	7.20 ± 0.00^{i}	8.10 ± 0.00^{h}	9.00 ± 0.00^{i}	9.00 ± 0.00^{g}	9.83 ± 0.13^{g}
TO51	19.05 ± 0.69^{a}	11.40 ± 0.52^{def}	12.00 ± 1.04^{fg}	12.00 ± 0.52^{e}	12.53 ± 0.72^{d}
TO52	19.20 ± 0.52^{a}	11.70 ± 0.00^{de}	12.60 ± 0.00^{ef}	12.60 ± 0.00^{d}	12.30 ± 0.69^{d}

Note: The value in the table is the average value of 3 repetitions ± standard deviation. Mean values in the same column followed by the same letters represent a statistically insignificant difference at the 95% confidence level ($P < 0.05$).

phenotypes, and their delineation is mainly based on genetic analysis. *Weissella cibaria* has been found in plant fermentation such as kimchi, cassava, mustard, and sourdough (LONVAUD-FUNEL, 2014). According to DI CAGNO & CODA (2014), each fruit and vegetable provides a unique environment in terms of microflora, and LAB accounts for a small fraction (2-4 logCFU/g) and is mainly affected by host vegetable species, temperature, and harvesting conditions. In addition, *W. cibaria* has been described to be able to produce GABA and isolated from various fermented food sources such as cheese, chutney, and khoji prepared from yam tuber (*Dioscorea* sp.), koozh, and kanji made from rice and millet Idli batter, buttermilk, and fermented beans (SIRAGUSA et al., 2007; DEVI et al., 2023). This indicated the potential application of *Weissella cibaria* in the production of GABA-enriched fermented tomato juice.

Effect of the dissolved solid concentration on tomato juice fermentation

Fermentation results for 24, 48, 72, and 96 h revealed two main trends in GABA content (Figure 4A). In treatments with 15 ºBrix and 20 ºBrix, GABA content peaked at 24 h (5.300 ± 0.292 mg/mL; 6.955 ± 0.000 mg/mL) and 72 h (9.185 ± 0.398 mg/mL; 9.019 ± 0.441 mg/mL) and decreased at 48 h (3.771 ± 0.292 mg/mL; 5.028 ± 0.389 mg/mL) and 96 h (3.325 ± 0.382 mg/mL; 8.170 ± 0.530 mg/mL). In the study by YOGESWARA et al. (2020), GABA production was also observed in MRS broth supplemented with 5% MSG (w/v) when culturing LAB strains isolated from food. The highest GABA concentrations were reported in two strains, IFK-10 and IFK-11, with the highest GABA concentrations being 2.68 and 2.06 mg/mL, respectively. These strains were identified as *Lactiplantibacillus plantarum* IFK-10 and *Pediococcus pentosaceus* IFK-11. The research also showed that GABA biosynthesis is stimulated when sufficient nutrients, GAD enzymes, and glutamate are available, whereas GABA degradation occurs when nutrient deficiencies are present, leading to the formation of GABA transaminase and alpha-ketoglutarate. Moreover, in the study by NAKATANI et al. (2022), when producing GABA-rich tomato juice using *Lactiplantibacillus plantarum* KB1253, isolated from Japanese pickles, it was found that the expression of the *GAD* gene and the decarboxylation activity of glutamic acid increased at low pH levels (3.0-3.5). This strain of *Lactiplantibacillus plantarum* KB1253 produced 41.0±1.1 mM GABA from glutamate in tomato juice under optimal fermentation conditions (pH 4.0; 20 °Brix). The authors also suggested that during the mid-phase of fermentation, as the bacteria start to deplete their nutrient supply, GABA transaminase enzymes and

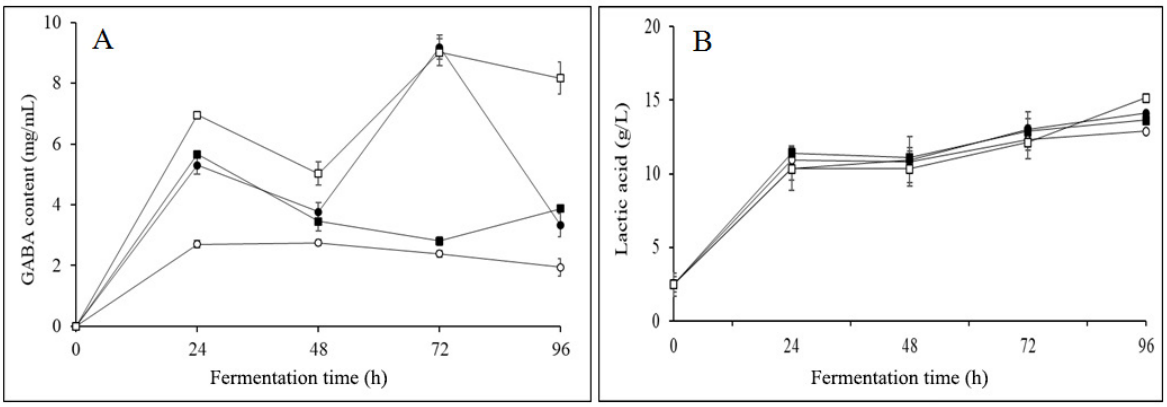

Figure 4 - Change of GABA content (A) and lactic acid content (B) produced during 24-96 h of tomato juice fermentation with *Weissella cibaria* TO42 (○: 5 °Brix; ■: 10 °Brix; ●: 15 °Brix; □: 20 °Brix).

Note: The fermentation broth was diluted with sterile deionized water 2, 4, 6 and 8 times to Brix of 5, 10, 15 and 20, respectively, before chromatography. The values in the table are the mean of 3 replicates ± standard deviation. Mean values in the same column followed by the same letters represent a statistically insignificant difference at the 95% confidence level ($P < 0.05$).

α-ketoglutarate are activated to break down GABA, generating glutamate and providing energy for the bacteria, resulting in a decrease in GABA concentration.

In experiments conducted at 5 ºBrix and 10 ºBrix, lower GABA levels were observed, and the fluctuations in GABA levels were less obvious. This is likely due to the low initial solute concentration and limited nutrient availability, resulting in a suboptimal growth rate for LAB. These findings are consistent with the research by WU & SHAH (2015) regarding the investigation of GABA production capabilities of nine LAB strains isolated from Kimchi, where MSG (0-70 g/L) was supplemented in a liquid MRS medium during a 72-h fermentation period. This study demonstrated that the variation in GABA levels in the fermentation environment is dependent on the glutamate concentration in the medium and the fermentation duration.

Conversely, the results showed that the lactic acid content in fermented tomato juice changed over time (Figure 4B). The lactic acid content at all solute levels tended to increase and reached the highest at 20 ºBrix at 96 h of fermentation at 15.15 ± 1.13 g/L. Tomatoes contain a small amount of sugar; therefore, adding sucrose improves the taste of the product and provides a source of carbon for LAB growth. The amount of lactic acid in fermented tomato juice and LAB depends on the strain, bacterial density, fermentation time, temperature, pH, and initial sugar content of the tomato juice. According to research by YOON et al. (2004), the lactic acid content in fermented tomato juice can range from 5 to 15 g/L after 24 to 48 h of fermentation at 30 ℃. However, the fermentation time is too long (96 h) leading to a significant reduction in the GABA content. As a result, the fermentation time of 72 h at 15 ºBrix will be suitable for fermentation of fermenting GABA-enriched tomato juice because the GABA and lactic acid contents recorded were 9.185 ± 0.398 mg/mL and 13.05 ± 1.56 g/L, respectively.

CONCLUSION

In conclusion, 15 lactic acid bacteria strains capable of GABA production were isolated from tomatoes. Among them, strain TO42, identified as *Weissella cibaria,* exhibited good and stable fermentation ability and was selected for further testing in tomato juice fermentation. The appropriate conditions for fermentation were identified to be 15 °Brix for 72 h, the GABA and lactic acid contents in the final product were attained at 9.185 ± 0.398 mg/mL and 13.05 ± 1.56 g/L, respectively. The findings reveal that the fermented GABA-enriched tomato juice will be a promising functional beverage which contains good sources of probiotic bacteria and bioactive compounds.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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