Versi Online: http://journal.ipb.ac.id/index.php/jtip
https://doi.org/10.6066/jtip.2025.36.2.195
Hasil Penelitian

Jurnal Teknologi dan Industri Pangan
Vol. 36(2): 195-207 Th. 2025 ISSN: 1979-7788
Terakreditasi Kemendikbudristek: 164/E/KPT/2021

Glutamic Acid Production by Lactic Acid Bacteria Isolated from Indonesian Fermented Food Salted Mustard Greens and Dangke Cheese

Silvana Nurulfauziyyah Indahsari, Siti Nur Jannah*, and Arina Tri Lunggani

Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia

Received October 15th 2024 / Revised June 3rd 2025 / Accepted June 16th 2025

ABSTRACT

Glutamic acid is an additive compound widely added to food to enhance the savory taste (umami). Lactic acid bacteria (LAB) are included in Generally Recognized as Safe (GRAS) and have the potential to produce various metabolite compounds, including glutamic acid, through fermentation. LAB can be isolated from salted mustard greens and dangke cheese. This study aimed to analyze the effect of LAB isolate types and fermentation time on the production of glutamic acid, glutamic acid profiling, and molecularly identify the LAB genus that produces the highest glutamic acid based on the 16S rRNA gene. The fermentation process of LAB was carried out using four selected isolates: D16, D15, S4, and S15, which were isolated from salted mustard greens and dangke cheese. Each isolate was incubated for five different incubation times: 0, 12, 24, 48, and 72 h. The identification of glutamic acid was carried out using the Thin Layer Chromatography (TLC) method, its quantification by spectrophotometry, and profiling by High-Performance Liquid Chromatography (HPLC). In addition, molecular identification of the highest-producing LAB isolate was conducted based on the 16S rRNA gene. The results showed that isolate S4 from salted mustard greens produced the highest glutamic acid after 48 h, with 670.05 mg/L and a total glutamic acid of 0.23% (w/w) based on HPLC results. Isolate S4 is known to be molecularly similar to the Pediococcus pentosaceus species. Local LAB isolates from salted mustard greens and dangke cheese can produce glutamic acid that can be used to enhance the taste of fermented foods.

Keywords: *dangke cheese, glutamic acid, HPLC, Pediococcus pentosaceus, salted mustard greens*

INTRODUCTION

Glutamic acid is an additive widely added to food to enhance the savory taste (umami). Glutamic acid in the form of salt is known as Monosodium Glutamate (MSG), and its consumption in Indonesia continues to increase yearly. According to Mahdi and Hidayat (2022), the potential production capacity of monosodium glutamate in Indonesia in 2026 is expected to reach 80,000 tons/year. In particular, China leads in the production and consumption of MSG worldwide, followed by several Southeast Asian countries, including Indonesia, Vietnam, and Thailand. This growth can be attributed to the increasing standard of living, cultural shifts, dietary changes, the development of the food processing industry, and population growth and urbanization (Thuy *et al.,* 2020).

Glutamic acid is a raw material used to produce MSG. MSG production is carried out considering various factors, including primary raw materials, price, temperature, pH, product conversion, purity, catalysts, and waste (Mahdi and Hidayat, 2022). MSG production is currently widely carried out through microbial fermentation processes because the cost of producing MSG through biosynthesis is low, the product purity is greater than 80%, and there is minimal waste (Mahdi and Hidayat, 2022). In addition, the microbes currently known for MSG production are *Corynebacterium glutamicum* and *Brevibacterium lactofermentum*. Although Gram-positive, part of their cell envelopes resemble those of Gram-negative bacteria because they have an outer lipid layer composed of mycolic acids, making them less susceptible to antibiotics (Ganguly, 2023; Zhao *et al*., 2015).

One way to contribute to industrial fermentation technology research is to explore the potential of LAB as a starter for glutamic acid production. LAB, which is classified in the Generally Recognized as Safe (GRAS) category, is non-pathogenic, safe for consumption, and tolerant to acid and bile, producing antimicrobial substances (Shehata *et al.,* 2016). In addition, LAB improves taste and nutrition, extends shelf life, and makes essential metabolites such as organic acids (lactic acid, acetic acid), vitamins, fatty

*Corresponding Author: E-mail: nurjannah.suroso@gmail.com

acids, peptides, and glutamic acid through fermentation (Wang *et al.,* 2021; Wang *et al.,* 2023). *Lactobacillus*, *Levilactobacillus brevis* PML1, *Lactiplantibacillus plantarum* 1058, and *Limosilactobacillus fermentum* 4–17 are types of lactic acid bacteria that can produce glutamic acid (Ghazanfari *et al.,* 2023).

Salted mustard greens and dangke cheese are examples of Indonesian fermented foods rich in nutrients, where the fermentation process involves LAB, which plays a role in glutamate synthesis. LAB isolates from salted mustard greens and dangke cheese, such as *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Pediococcus cerevisiae*, have the potential to produce various beneficial metabolites (Chun *et al.,* 2017). Although various microbes can be present in fermented products, traditional fermented foods often have a unique microbial profile for each product (Baek *et al.,* 2024).

The selection of LAB strains that produce glutamic acid and elucidation of their metabolic pathways remain to be explored. Therefore, this study aimed to examine the total amino acids, including glutamate, obtained from the fermentation of the highest glutamic acid-producing LAB isolates, explain the possible metabolic pathways involved, and molecularly identify the LAB genus producing the highest glutamic acid based on the 16S rRNA gene. The medium used in this study was De Man, Rogosa, and Sharpe (MRS) broth supplemented with 0.5% citrate (%w/v). The research results are expected to aid in selecting the optimal LAB strain from native microorganisms that are well-adapted, competitive, and possess high metabolic capacity.

MATERIALS AND METHOD

Materials

The cultures and materials used in the study included four LAB isolates: S4 and S15 (from salted mustard greens), and D15 and D16 (from dangke cheese). All isolates were obtained from the Biotechnology Laboratory of Diponegoro University. Other materials included De Man, Rogosa, and Sharpe (MRS) broth media (Himedia, Germany), MRS agar media (Himedia, Germany), citric acid (Merck, Germany), n-butanol (Merck, Germany), acetic acid (Merck, Germany), glutamic acid (Merck, Germany), ninhydrin (Merck, Germany), InstaGene Matrix, MyTaq® master mix, primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), agarose gel (Merck, Germany), staining gel (Red Safe), DNA ladder, and Tris-acetate-EDTA (TAE) buffer 1X.

Rejuvenation and characterization of LAB isolates

All LAB isolates were rejuvenated using the streak plate method in quadrants on MRS agar supplemented with $CaCO_3$ and then incubated at 37 °C for 24−48 h (Sasmita *et al.,* 2018). Pure LAB isolates were characterized macroscopically and microscopically with Gram staining. After that, the catalase test and fermentation-type test were continued (Abdullah *et al*., 2021; Ismail *et al.,* 2017).

LAB growth curve preparation

Single colonies from each LAB isolate from salted mustard greens and dangke cheese were inoculated into 15 mL of NaCl each and equated to the McFarland 0.5 standard. The next stage was to create a growth curve by taking 5% inoculum and then inoculating it into 80 mL of MRS broth while adding 0.5% citrate (%w/v). Furthermore, the inoculated broth was incubated at room temperature for 72 h. LAB growth was observed by observing the absorbance value at 12, 24, 48, and 72 h at 600 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280, Shimadzu Corp., Japan).

Glutamic acid production from LAB isolates

The preparation of LAB fermentation medium for glutamic acid production refers to Lee *et al*. (2021), namely by inoculating 5% of each LAB inoculum into 80 mL of MRS broth media, adding 0.5% citrate (v/v). After that, it was incubated for 0, 12, 24, 48, and 72 h at room temperature at a speed of 150 rpm. MRS broth supplemented with 0.5% citrate without adding LAB isolates was used as a control.

The samples consist of all four LAB isolates (S4, S15, D15, and D16) and the control, which were incubated under the same time variations (0, 12, 24, 48, and 72 h at room temperature). All observed parameters (pH, total lactic acid, glutamic acid identification, and spectrophotometric quantification) were applied to every sample at each time point. HPLC and molecular identification analyses were conducted only for isolate S4 at 48 h, as it produced the highest glutamic acid concentration.

pH measurement

Value of pH was observed to determine the level of acidity produced by LAB fermentation in each treatment of different LAB isolates after an incubation period of 0, 12, 24, 48, and 72 h using a universal pH indicator (Merck, Germany).

Measurement of total lactic acid

Samples were taken from all LAB isolates and the control at all incubation times (0, 12, 24, 48, and 72 h). A one mL sample of each culture was diluted 30 times in distilled water. Then, the sample was added with 2−3 drops of 1% phenolphthalein indicator

and titrated with a standardized 0.1 N NaOH solution. The change of the solution to pink marked the endpoint of titration.

Identification of glutamic acid by thin layer chromatography (TLC)

Identification of glutamic acid was carried out by thin-layer chromatography (TLC) of silica gel plates (Macherey Nagel, Macherey-Nagel GmbH & Co. KG, Germany), referring to the modified method (Ghazanfari *et al*., 2023). The supernatant of the fermentation culture solution was obtained by centrifugation at 10,000 rpm for 15 min. After that, 80% ammonium sulfate was added to the supernatant and centrifuged (Ohaus Frontier™ 5718R, Ohaus Instruments Co. Ltd., China) again at 10,000 rpm at 4 °C for 20 min. The supernatant was taken, and the analysis was carried out using a mobile phase of n-butanol: acetic acid: water (1:2:4 v/v). Pure glutamic acid was used as the standard. To visualize the separated compounds, the plate was sprayed with 0.5% ninhydrin solution in 96% ethanol and then placed in an oven at 90 °C for 10 min.

Total glutamic acid measurement by spectrophotometry

Total glutamic acid measurement was carried out by spectrophotometry (Maslami *et al.,* 2023). As much as 1 mL of supernatant from lactic acid bacteria culture was taken, then 1 mL of 0.1% ninhydrin was added and vortexed. The sample solution was heated in boiling water for 5 min and then cooled with water. Then, the absorbance of each sample was read at 570 nm. The absorbance values were converted into glutamic acid concentrations using a standard curve prepared with pure glutamic acid.

Total amino acids by High-Performance Liquid Chromatography (HPLC)

The S4 isolate, incubated for 48 h, was selected for further analysis due to its highest total glutamate concentration, as determined by spectrophotometry analyses. The analysis was conducted at the Palm Oil Research Center (PPKS) laboratory in Bogor. The analysis involved an HPLC (Shimadzu SCL-10A/ Shimadzu CBM-20A, Shimadzu Corp., Japan) test to determine the type of amino acids present in the sample solution, including total glutamate. Amino acid analysis was performed using a Thermo Scientific ODS-2 Hypersil column, with OPA derivatization and fluorescence detection.

Molecular identification of LAB isolates with the 16S rRNA gene

Isolate S4 was selected for molecular identification because it produced the highest total glutamate compared to other isolates. DNA isolation was performed using the InstaGene Matrix protocol. Quantitative DNA analysis was performed using a nanodrop tool (Thermo Fisher Scientific, USA). PCR amplification of the 16S rRNA gene was performed using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Amplification began with the preparation of a 50 *µ*L PCR mix consisting of 25 *µ*L MyTaq™ Mix, 2 *µ*L primer 27F, two *µ*L primer 1492R, two *µ*L (200 ng) DNA template, and ddH_2O up to 50 *µ*L. Furthermore, PCR (Labnet International Inc., USA) was carried out with a pre-denaturation stage at 95 °C for three min, then lasted for 35 cycles, including denaturation at 95 °C for 45 sec, primer attachment at 54 °C for one min, and extension at 72 °C for 90 sec. After that, the elongation phase was carried out at 72 °C for 10 min and the cooling phase at 4 °C for an unlimited time (Mardiana *et al*., 2020). The amplified DNA was electrophoresed with 1% agarose gel for 25 min at 100 volts. The electrophoresis gel was soaked in a gel staining solution. The marker used was five *µ*L of a kb DNA ladder. After electrophoresis, the resulting bands were observed by moving the agarose gel to a UV transilluminator. The sequencing results were edited using BioEdit and then Basic Local Alignment Search Tool (BLASTed) accessed through the National Center for Biotechnology Information (NCBI) website. After that, 10 BLAST sequences with the highest homology and one outgroup sequence with the same family were downloaded in FASTA format. Phylogenetic tree construction was performed using MEGA (Version 10.2), Molecular Evolutionary Genetics Analysis (MEGA) Software, USA.

Data analysis

Data analysis began with a normality test and a homogeneity test. If the results are normal and homogeneous, a two-way analysis of variance (ANOVA) was continued. Then, if the results obtained were significantly different between samples using SPSS Statistics (Version 26.0, IBM Corp., USA), the statistical analysis was continued with the Tukey test at a 95% confidence level.

RESULTS AND DISCUSSION

Characteristics of LAB isolates

The macroscopic morphological characteristics of lactic acid bacteria isolated from salted mustard greens and dangke cheese were creamy white colonies, round with flat edges, and clear zones on MRSA media supplemented with 0.5% $CaCO_3$. The resulting clear zones indicate that LAB isolates could produce acidic compounds. The macroscopic morphological characteristics of LAB isolated from salted mustard and dangke cheese can be seen in Table 1 and Figure 1.

Table 1. Macroscopic morphological characteristics of LAB isolated from salted mustard greens and dangke cheese

Isolate	Origin	Colony Morphological Characteristics					
		Shape	Color	Edge	Elevation	Colony Appearance	Optical Properties
S4	Salted mustard greens	Round	Creamy White	Flat	*Convex*	Glossy	Opaque
S15	Salted mustard greens	Round	Creamy White	Flat	*Convex*	Glossy	Opaque
D15	Dangke cheese	Round	Creamy White	Flat	*Convex*	Glossy	Opaque
D16	Dangke cheese	Round	Creamy White	Flat	*Convex*	Glossy	Opaque

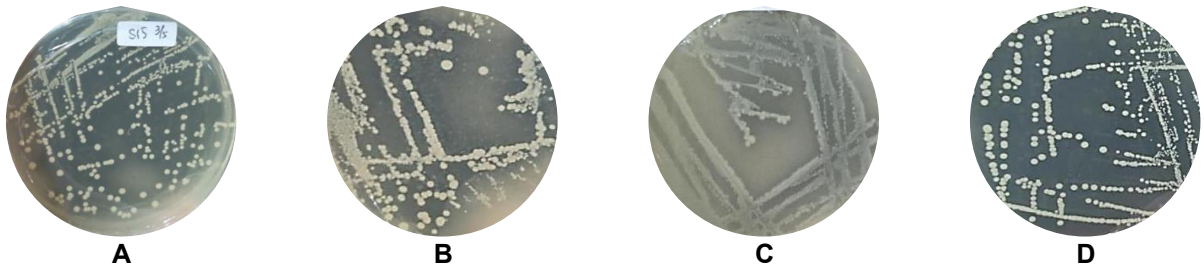

Figure 1. Formation of a clear zone by LAB isolates on MRSA + 0.5% $CaCO_3$ media; A= Isolate S15, B= Isolate S4, C= Isolate D16, D= Isolate D15

According to Goa *et al*. (2022), LAB have the characteristics of a round shape and a cream color. LAB isolates isolated from tempeh probiotic drinks have the characteristics of small to medium round colonies with flat edges, convex elevations and produce clear zones on MRSA media supplemented with 5% $CaCO_3$. Calcium carbonate ($CaCO_3$) has buffer activity and is an initial screening for lactic acid bacteria. The lactic acid compound produced by LAB will react with $CaCO_3$, so a clear zone appears around the colony after 48−72 h (Aýun *et al*., 2023).

The microscopic characteristics results show that the LAB isolates from salted mustard greens and dangke cheese are a group of Gram-positive bacteria. Identification of LAB isolates from salted mustard and dangke cheese can be seen in Table 2.

LAB growth curve

All LAB isolates from salted mustard greens and dangke cheese were grown in MRSB media supplemented with 0.5% citrate (w/v) and incubated for 72 h at room temperature. LAB growth can be seen in Figure 2. The growth phase of LAB can be observed starting from the lag, log, and stationary phase (Gonzalez and Aranda, 2023). Isolates S4, S15, and D15 showed adjacent lag phases in the first hour of incubation. Then, isolates S4, S15, and D15 showed log phases at 2 to 48 h incubation. The stationary phase of isolates S4, S15, and D15 began at 48 to 72 h of incubation. Isolate D16 showed a lag phase at 0 to 12 h, a log phase at 12 to 24 h, and a stationary phase at 24 to 72 h of incubation. The highest growth of isolates S4, S15, and D15 occurred at 48 h, with absorbance values at 600 nm of 2.03, 2.24, and 2.27, respectively. In contrast, isolate D16 reached its highest growth at 12 h of incubation, with an absorbance of 2.17.

During the growth phase, primary and secondary metabolites are produced (Sheikh *et al.,* 2024). Primary metabolites are produced in the trophophase, which is the phase where microorganisms have sufficient nutrients or begin to be synthesized and produced in the early log phase, and production reaches its optimum level in the late log phase (Azizah *et al*., 2023; Maldonado-Ruiz *et al.*, 2024). The idiophase is when secondary metabolites are produced, when exponential growth ends and enters the early stationary phase (Maldonado-Ruiz *et al.,* 2024; Sheikh *et al.,* 2024). The trophophase, characterized by balanced microbial growth conditions, produces essential primary metabolites (such as vitamins, amino acids, and nucleosides) and end products of primary metabolism (including lactic acid, ethanol, and acetone) (Ramesha and Deeksha, 2019). At the same time, the idiophase involves the production of secondary metabolites such as antibiotics, pigments, and enzymes (Fachrial *et al.,* 2025; Guryanov and Naumenko, 2024; Ramesha and Deeksha, 2019).

Referring to previous research, LAB isolates from fermented boza, as reported by Gökmen *et al*. (2024), showed an exponential phase until 18–20 h of incubation. The stationary phase occurred after 20 h. In another study, as reported by Azhara *et al*. (2022), LAB isolates from spontaneous fermentation of robusta coffee beans entered a lag phase at 0−3 h of incubation with colonies ranging from 6.8−6.9 log CFU/g. The exponential phase occurred at 3 to 72 h of fermentation, with colonies ranging from 6.9 to 9.5 log CFU/g.

Table 2. Identification of LAB isolated from salted mustard and dangke cheese

Isolate	Origin	Microscopic Characteristics		Catalase Test	Fermentation Type Test
		Shape	Gram		
S4	Salted mustard greens	Cocci	+	Negative	Homofermentative
S15	Salted mustard greens	Cocci	+	Negative	Homofermentative
D15	Dangke cheese	Cocci	+	Negative	Homofermentative
D16	Dangke cheese	Rod	+	Negative	Heterofermentative

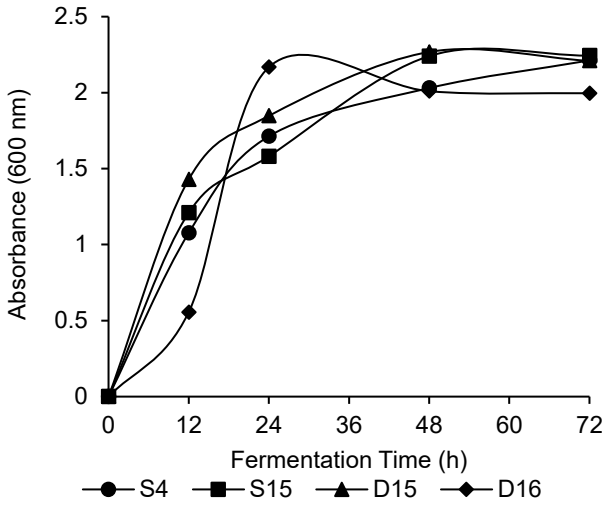

Figure 2. Growth activity of LAB isolates on MRS broth media + 0.5% citrate (%w/v). Absorbance at 600 nm; fermentation time (h)

pH and total lactic acid

The results showed that the pH of the LAB culture from salted mustard greens and dangke cheese decreased after fermentation for 12 h. The pH value at the beginning of fermentation ranged from 5 to 4. Evidence from previous research showed *dadih* fermented with *Pediococcus acidilactici* BK01 isolate reduced the pH from 5.20 to 5.10 after fermentation for 20 h (Melia *et al.,* 2020). Pangestika *et al*. (2018) also stated that the pH value of the *Lactobacillus acidophillus* fermentation culture in media containing D-fructose decreased by ±3% when compared to media without D-fructose. Furthermore, Rahmayetty *et al*. (2022) reported that a significant decrease in pH occurred after LAB fermentation in molasses media for 24 h.

The total lactic acid produced by LAB isolates from salted mustard and dangke cheese increased with increasing fermentation time. The total amount of lactic acid produced during fermentation presented in Table 3.

The increase in total lactic acid was due to the increasing number of LAB colonies, so the total lactic acid produced also increased. The S4 isolate yielded the highest total lactic acid content after 72 h, at 2.67%. This study aligns with the research of Rahmayetty *et al*. (2022), which reported that LAB fermentation in molasses media produced the highest lactic acid concentration at 72 h of 23.1 ppm. *Pediococcus acidilactici* BK01 produced 0.61% lactic acid after fermentation for 20 h (Melia *et al.,* 2020). LAB utilizes carbon sources in the media to be converted into lactic acid compounds. During fermentation, bacteria convert carbon sources, such as sugar, into lactic acid and energy to form new cells. The decrease in total lactic acid is due to the side effects of the subsequent reaction, which converts lactic acid into other compounds (Rahmayetty *et al.,* 2022).

The results of the statistical analysis test show that the total lactic acid data are normally distributed and homogeneous. This result is supported by the Shapiro-Wilk and Levene test significance values of 0.110 and 0.113, respectively, which are greater than 0.05. In addition, statistical analysis also shows no interaction between fermentation time and the type of LAB isolate in determining total lactic acid, as indicated by a Sig. Value at 0.245, greater than 0.05. However, the significant difference in total lactic acid was less than 0.05 based on the fermentation time factor variable and the type of LAB isolate factor.

Identification of glutamic acid by TLC

The glutamic acid identification test results showed that glutamic acid could be successfully identified from all samples. This result is indicated by the Rf value of the glutamate standard (0.3), which matches the Rf value of the sample under the same chromatographic conditions. This match confirms that the method used was able to separate glutamic acid from other compounds. The Rf value represents the ratio between the distances traveled by the solute and the solvent (mobile phase) from the origin on TLC plate (Guadarrama-Pérez *et al*., 2024). Reported Rf values from other studies may vary depending on the stationary and mobile phases used; for example, Hussein *et al*. (2021) reported an Rf of 0.51 for urine samples, and Tunio *et al*. (2022) reported 0.076 for glutamate, both obtained under different chromatographic conditions.

Glutamate compounds can be observed on TLC. The standard solution used is pure glutamic acid at a concentration of 700 ppm. Figure 3 shows the identification of glutamate with TLC. The TLC results in Figure 3 confirmed the presence of glutamate in the fermentation broth of several isolates, as indicated by the appearance of purple spots with Rf values matching the glutamate standard.

Table 3. Percentage of total lactic acid (%) produced by LAB isolated from salted mustard greens and dangke cheese

Isolate	Total Lactic Acid (%)				
	0 h	12 h	24 h	48 h	72 h
S4	1.02±0.31^{a}	1.61±0.78^{ab}	2.03±0.53^{ab}	2.28±0.38^{ab}	2.67±0.44^{b}
S15	0.95±0.21^{a}	1.50±0.64^{ab}	1.84±0.71^{ab}	2.16±0.39^{ab}	2.29±0.20^{b}
D15	1.02±0.31^{a}	1.80±0.63^{a}	2.19±0.37^{a}	2.16±0.38^{a}	2.13±0.14^{a}
D16	0.99±0.32^{a}	1.21±0.22^{a}	1.73±0.32^{a}	1.76±0.28^{a}	1.86±0.46^{a}
Control	1.08±0.26^{a}	1.08±0.26^{a}	1.08±0.26^{a}	1.08±0.26^{a}	1.08±0.26^{a}

Note: Different notations in the same row indicate significant effects (p<0.05) in the Tukey test. The control was MRSB + citrate media without the addition of BAL isolates. Data are presented as mean ± standard deviation (SD) from three independent replicates (n= 3)

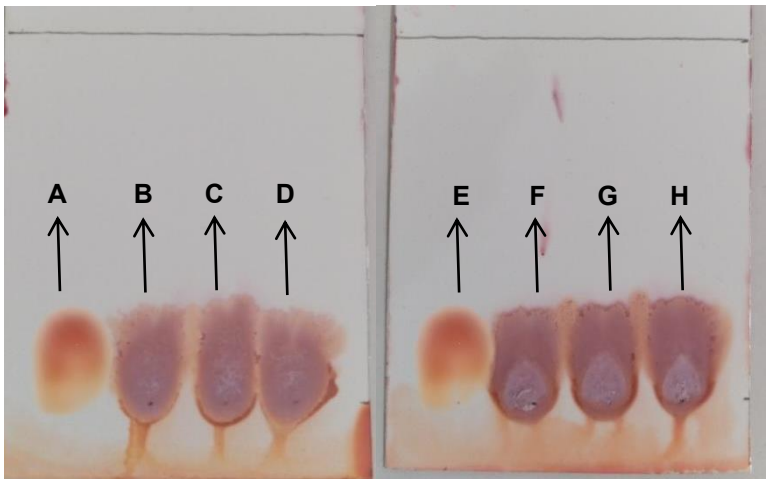

Figure 3. Identification of glutamate by TLC: A), E) Standard control of glutamate 700 ppm; Supernatant from the culture of isolate D16 after fermentation for 24 h (B), 48 h (C), 72 h (D); F) Isolate S4 after fermentation for 48 h; G) Isolate S15 after fermentation for 48 h; H) Isolate D15 after fermentation for 48 h

All isolates produced visible glutamate spots at 48 h, suggesting this time point may correspond to peak glutamate production. However, the intensity varied, possibly due to differences in metabolic activity or growth phase. These findings indicate that glutamate biosynthesis by lactic acid bacteria is time-dependent and strain-specific. Each isolate exhibited the most consistent and intense glutamate production over time, particularly at 48 h of fermentation, indicating its potential as a promising candidate for glutamate biosynthesis in future applications. Previous studies by Maslami *et al*. (2018) showed that out of 17 LAB successfully isolated from budu fish, only 16 isolates could produce glutamic acid. The highest glutamic acid production was observed in isolate IB.9, which produced 13.03 mg/mL after 72 h of incubation. Meanwhile, Ghazanfari *et al*. (2023) also detected glutamate compounds produced by LAB isolates fermented with media containing food waste, as determined by the TLC method. The difference in glutamic acid production of each isolate was due to the different abilities of each LAB strain (Maslami *et al*., 2018). Several genera of bacteria that can produce glutamate include *Brevibacterium*, *Corynebacterium*, *Microbacterium*, and *Micrococcus* (Shangguan *et al.,* 2023).

Total glutamic acid by spectrophotometry

The glutamic acid produced by LAB isolates from salted mustard and dangke cheese showed no difference among treatments. The amount of total glutamic acid produced during fermentation presented in Table 4. The maximum amount of total glutamic acid was produced by isolates S4, S15, and D15 at 48 h, and isolate D16 at 72 h. The addition of citrate to the MRSB medium triggers glutamate production. There were five pathways to form glutamic acid, derived from the following precursors: citrate, glutamine, histidine, proline, and putrescine. Citrate compounds can be synthesized from pyruvate in the *L. lactis* strain IL1403, as this bacterium possesses the necessary genes to convert citrate into glutamate (Lee *et al.,* 2021). Glutamic acid synthesis is attributed to increase regulation of genes encoding citrate synthase (*cs*), isocitrate dehydrogenase (*icdh*), and glutamate dehydrogenase (*gdh*), and decreased regulation of genes encoding the oxoglutarate dehydrogenase complex (*odhc*) (Shangguan *et al.,* 2023).

Table 4. Total glutamic acid (mg/L) produced by LAB isolated from salted mustard greens and dangke cheese

Isolate	Total Glutamic Acid (mg/L)				
	0 h	12 h	24 h	48 h	72 h
S4	632.17±14.40[a]	665.40±55.63[a]	651.06±29.84[a]	670.05±51.44[a]	660.35±30.48[a]
S15	646.11±37.05[a]	656.31±40.54[a]	648.84±30.14[a]	658.54±21.89[a]	642.47±11.52[a]
D15	648.84±30.14[a]	644.40±22.16[a]	640.35±16.12[a]	658.54±21.89[a]	646.11±13.41[a]
D16	641.36±30.06[a]	647.12±17.89[a]	645.10±12.27[a]	649.24±21.10[a]	650.05±7.69[a]
Control	636.82±20.11[a]	642.47±20.80[a]	639.44±16.06[a]	642.58±19.33[a]	641.36±18.28[a]

Note: The same notation in the same row indicates no significant difference in the Tukey test. The control was MRSB + citrate media without the addition of BAL isolates. Data are presented as mean ± standard deviation (SD) from three independent replicates (n= 3)

Several factors, such as fermentation media and the type of bacterial isolate used, cause differences in total glutamic acid production by LAB. Several strains of lactic acid bacteria fermented in MRS media with the addition of 20% glycerol (v/v) produced different total glutamic acid. The highest glutamate production was found in *P. acidilactici* UP-1, and the lowest was in *P. pentosaceus* UP-2 (Toe *et al*., 2019). M17 broth media, given different precursors, produced different total glutamate levels. Media enriched with 0.5% citrate precursor had the highest total glutamate production by *Lactococcus lactis* (108.42 pmol/μL), whereas media enriched with 0.5% glutamine had the lowest glutamate production (60.65 pmol/μL) (Lee *et al*., 2021).

The results of the statistical analysis test showed that the total glutamic acid data were normally distributed and homogeneously distributed. This result can be seen from the Sig—Shapiro-Wilk and Levene test values at 0.280 and 0.051, giving higher value than 0.05. In addition, statistical analysis showed no interaction between fermentation time and the type of LAB isolate in determining total glutamic acid, as indicated by a significance value of 1, which is greater than 0.05. In addition, there was no difference in total glutamic acid (Sig.)>0.05 based on the fermentation time factor variable and the type of LAB isolate factor.

Total amino acids by HPLC

Isolate S4 was selected for further testing based on the results of the spectrophotometric analysis, which showed that this isolate produced the highest total concentration of glutamate, 670.05 mg/L, among all tested isolates. Therefore, S4 was chosen for subsequent confirmation and quantification using HPLC to validate and further characterize its glutamate production capacity. Selecting the isolate with the highest glutamate yield is critical in screening for potential candidates for industrial or biotechnological applications. The present findings showed that 15 amino acids were identified from the fermentation sample of isolate S4. Table 5 shows the total amino acid produced by isolate S4 after fermentation for 48 h.

Amino acids are the essential components of protein and are substrates for protein synthesis. Amino acids consist of essential amino acids and non-essential amino acids. Essential amino acids are amino acids that cannot be synthesized de novo, so they are obtained from outside the body, such as food (Gwin *et al*., 2020). In contrast, non-essential amino acids are amino acids that can be produced in the body. Non-essential amino acids consist of alanine, arginine, aspartic acid, asparagine, cystine, cysteine, glutamic acid, glutamine, glycine, proline, and serine (Kamble *et al*., 2021).

Table 5. Total amino acids analyzed by HPLC in isolate S4 after 48 h of fermentation

Parameter	Total (%w/w)
Aspartic acid	0.15
Threonine	0.05
Serine	0.09
Glutamic	0.23
Glisine	0.07
Alanine	0.08
Valine	0.21
Methionine	0.26
Ileusine	0.05
Leusine	0.06
Tyrosine	0.10
Phenylalanine	0.08
Histidine	1.16
Lisine	0.08
Arginine	1.24
Total amino acid	3.91

Based on Table 5, it is known that there are seven types of essential amino acids produced by isolate S4, namely histidine, leucine, threonine, valine, methionine, isoleucine, phenylalanine, and eight non-essential amino acids, namely serine, aspartic acid, arginine, lysine, glutamate, glycine, alanine, and tyrosine. Arginine is the amino acid with the highest content, followed by histidine, methionine, and glutamate. Amino acids have various health benefits in addition to their role in protein synthesis. Amino acids are components of proteins and natural organic compounds containing nitrogen, which are essential for health. Amino acids show a broad spectrum of biological activities, including their ability to activate NRF2, a transcription factor that regulates endogenous antioxidant responses (Egbujor *et al*., 2024).

In addition to protein synthesis, amino acids play a role in the adaptation of LAB to their environment. Amino acids (especially glutamine, glutamic acid, and arginine) adapt lactic acid bacteria to acidic environments. NH_3 synthesized during amino acid deamination can increase the pH value inside and outside the cell, thereby protecting cells from acid stress (Papadimitriou *et al*., 2016). As a bioactive molecule, glycine regulates gene expression for cytoprotection, protein configuration and activity, and other critical biological processes, including glutathione synthesis (Quintanilla-Villanueva *et al*., 2024). The amino acids L-cysteine, L-alanine, and L-histidine can function as nitrogen sources, enhance microbial growth, and act as electron transporters in microbial biochemical reactions (Helmstetter *et al.,* 2019; Liu *et al.,* 2022). D-leucine and D-methionine can act as signaling molecules to regulate cell wall biogenesis and biofilm integrity, trigger microbial biofilm disintegration at low concentrations to regulate bacterial population distribution and affect the surrounding ecosystem (Liu and Chen, 2018; Liu *et al.,* 2022; Wang *et al.,* 2022).

The content of L-aspartate and glycine correlates with the yield of lactic acid in anaerobic fermentation (Song *et al.,* 2023). Cysteine has been shown to increase the yield of lactic acid from food waste by up to 13%. The addition of cysteine increases the activity of α-glucosidase and the rate of hydrolysis and, at the same time, increases the abundance of *Lactobacillus* in the acidification stage and lactate dehydrogenase, all of which also support lactic acid production. However, adding valine has been shown to reduce the yield of lactic acid by 18%, and the results imply that valine appears to inhibit carbohydrate conversion (Zhou *et al.,* 2024).

Amino acids can also play a role in the taste of fermented foods. Free amino acids produced through proteolysis can be converted into various compounds that play a role in taste, including ammonia, amines, aldehydes, phenols, indoles, and alcohols, all of which can contribute to the taste of yogurt (Chen *et al.,* 2017). L-glycine and L-glutamic acid significantly reduced the production of intracellular reactive oxygen species and the levels of malondialdehyde and carbonylated proteins. They simultaneously increased the ATP levels in *P. pentosaceus* R1 bacteria under H_2O_2-induced stress. L-glycine and L-glutamic acid also suppressed bacterial membrane deformation and cell damage. In addition, L-glycine and L-glutamic acid significantly increased the activities of superoxide dismutase and glutathione peroxidase and increased the total antioxidant capacity of bacteria. Thus, L-glycine and L-glutamic acid can alleviate oxidative stress caused by H_2O_2 through direct antioxidant effects and enhance the activities of bacterial antioxidant enzymes (Zhang *et al*., 2022).

Glutamate is associated with umami taste. It is used in savory foods such as spices, herbs, meat products, soups, and broths to enhance flavor and deliciousness (Zhang *et al*., 2017). In enterocytes, glutamate is transaminated into various amino acids, mainly alanine and proline, aspartate, and citrulline (Cynober, 2018).

Molecular identification of LAB isolates with the 16S rRNA gene

Isolate S4 was molecularly identified using the 16S rRNA gene and achieved a purity of 2.13, with an A260/280 ratio of 1.32 and a total DNA concentration of 1326.3 ng/μL. The purity value in the range of 1.8−2.0 indicates that the DNA sample has good purity. Purity results lower than 1.8 indicate that the concentration of innate protein is relatively high. In comparison, if the purity results are higher than 2.0, the DNA concentration is relatively high (Syahputra *et al*., 2016).

The 16S rRNA gene amplification results were analyzed by electrophoresis and using a 1 kb marker ladder. The results showed that isolate S4 could produce DNA bands with a length range of 1500 bp in the agarose gel electrophoresis results. The electrophoresis results are shown in Figure 4. The 16S rRNA gene is approximately 1,500 to 1,600 base pairs (bp) in length in most bacteria. It consists of nine variable regions that allow taxonomic identification of the microbial community (Yeo *et al*., 2024).

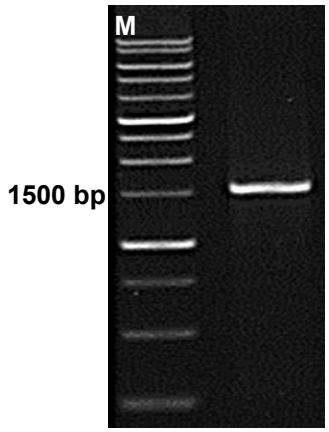

Figure 4. Visualization of electrophoresis results of isolate S4 sample with 1 kb DNA ladder (M)

The phylogenetic tree of isolate S4 is presented in Figure 5. The phylogenetic tree showed that isolate S4 had a bootstrap value of 100 with the species *Pediococcus pentosaceus* strain HBUAS62112. The bootstrap value in a phylogenetic tree indicates how often the same branch is observed across 100 repetitions of phylogenetic tree creation using resampled data sets (Ojha *et al.,* 2022).

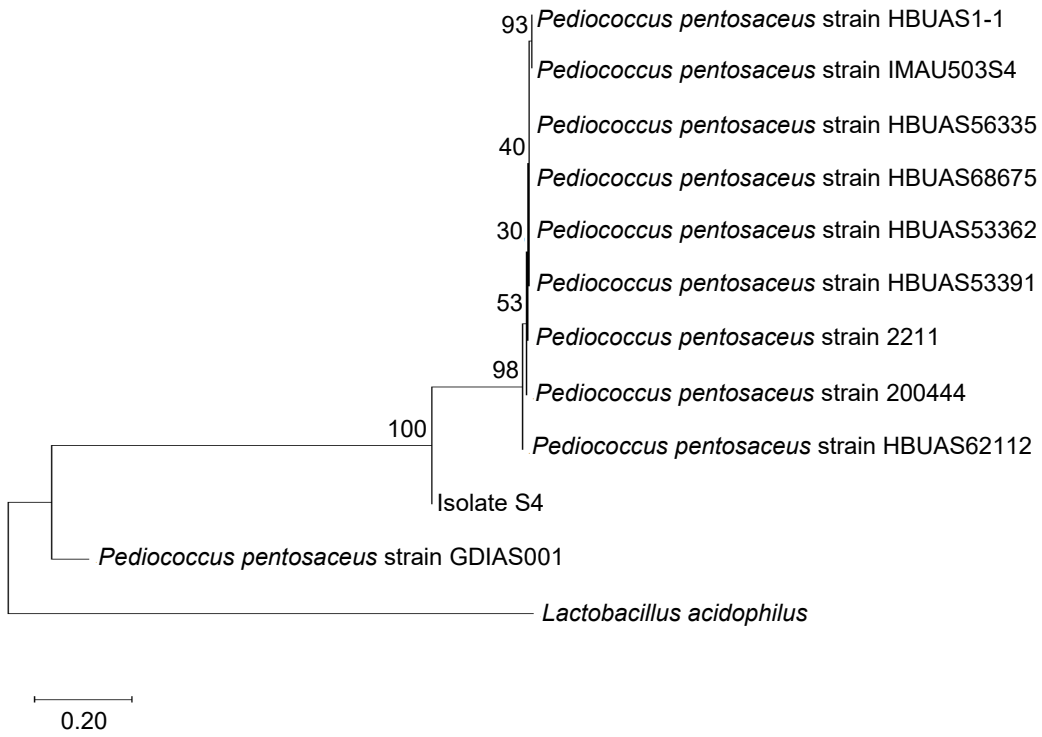

Figure 5. Phylogenetic tree of isolate S4 with Neighbor-Joining (NJ) analysis and Bootstrap phylogeny test, 1000 replicates

A bootstrap threshold above 70% is commonly used to indicate well-supported branches in a phylogenetic tree (Lemoine and Gascuel, 2024). Isolate S4 was Gram-positive, catalase-negative, and had a coccus-shaped morphology. *Pediococcus pentosaceus* is a lactic acid bacteria belonging to the Lactobacillaceae family, genus *Pediococcus*, with coccus-shaped characteristics, and is a non-motile facultative anaerobic Gram-positive bacteria (Chen *et al.,* 2020). In another study, *Pediococcus pentosaceus* can be isolated from fermented sausages from the Basilicata region (Southern Italy) (Tathode *et al.,* 2024).

Pediococcus strains are used as natural preservatives to extend the shelf life and hygienic food quality due to their unique properties in producing bacteriocins. These bacteria work against bacteria that cause food contamination, such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Clostridium botulinum*. *Pediococcus pentosaceus* can also be found in the intestines of marine shrimp from the Mediterranean Sea (Zaghloul and Halfawy, 2024). A study by Toe *et al*. (2019) showed that glutamate can be produced by several strains of *Pediococcus pentosaceus* from Malaysian fermented food. *Pediococcus pentosaceus* UL-6, UP-2, UB-8, and UL-2 each produced glutamate of 38.87 mg/L, 98 mg/L, 45.81 mg/L, and 66.64 mg/L, respectively.

CONCLUSION

Glutamic acid production could be influenced by LAB isolate type and fermentation time, with isolate S4 from salted mustard greens producing the highest glutamate concentration at 48 h of fermentation. Amino acid profiling confirmed the presence of glutamic acid along with other amino acids, including arginine, histidine, methionine, and valine, produced during fermentation. Molecular identification based on 16S rRNA gene analysis revealed that isolate S4 had similarities with the species *Pediococcus pentosaceus*. Based on the citrate-supplemented fermentation medium, it is likely that glutamic acid was synthesized through the conversion of α-ketoglutarate via the citrate metabolism pathway. These results highlight *P. pentosaceus a*s a promising native LAB strain, and emphasize the importance of selecting strains based on both glutamate yield and their broader amino acid profile to enhance the nutritional and functional value of fermented products. Further analysis, such as variations in fermentation substrates, is needed to determine the production capacity of other LAB isolates, and molecular identification tests of the gdh gene are required to determine the genomic pathway of glutamic acid biosynthesis in LAB.

ACKNOWLEDGEMENT

The author would like to express their gratitude to the Ministry of Education, Culture, Research, and Technology's *Unggulan* Scholarship for providing educational funding for the first author so that the author can continue her studies to the master's level.

REFERENCES

Abdullah, D., Poddar, S., Rai, R. P., Purwati, E., Dewi, N. P., & Pratama, Y. E. (2021). Molecular identification of lactic acid bacteria: An approach to sustainable food security. *Journal of Public Health Research*, *10*(s2), jphr.2021.2508. https://doi.org/10.4081/jphr.2021.2508

Aýun, Q., Muthiáh, S. N., & Sukmalara, D. (2023). Potensi bakteri asam laktat (BAL) dari jus tempe sebagai kandidat probiotik. *Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi*, *8*(2), 171–177. https://doi.org/10.36722/sst.v8i2.1673

Azhara, I., Rais, M., Sukainah, A., & Putra, R. P. (2022). Isolasi dan identifikasi bakteri asam laktat pada fermentasi spontan biji kopi robusta asal Bantaeng. *Jurnal Teknologi Pertanian*, *23*(1), 49–60. https://doi.org/10.21776/ub.jtp.2022.023.01.5

Azizah, S. N., Rosida, R., Hidayah, A. N., & Dwijayanti, A. R. (2023). Lactic acid bacteria administration from Jember tempeh (Indonesia) as a probiotic candidate in intestinal physiology and histology of mice strain Balb-C. *Biodiversitas Journal of Biological Diversity*, *24*(12). https://doi.org/10.13057/biodiv/d241258

Baek, J. H., Han, D. M., Choi, D. G., & Jeon, C. O. (2024). Unraveling the carbohydrate metabolic characteristics of *Leuconostoc mesenteroides* J18 through metabolite and transcriptome analyses. *Food Chemistry*, *435*, 137594. https://doi.org/10.1016/j.foodchem.2023.137594

Chen, C., Zhao, S., Hao, G., Yu, H., Tian, H., & Zhao, G. (2017). Role of lactic acid bacteria on yogurt flavour: A review. *International Journal of Food Properties*, *20*(sup1), S316–S330. https://doi.org/10.1080/10942912.2017.1295988

Chen, T., Wang, L., Li, Q., Long, Y., Lin, Y., Yin, J., & *et al*. (2020). Functional probiotics of lactic acid bacteria from Hu sheep milk. *BMC Microbiology*, *20*, 228. https://doi.org/10.1186/s12866-020-01920-6

Chun, B. H., Kim, K. H., Jeon, H. H., Lee, S. H., & Jeon, C. O. (2017). Pan-genomic and transcriptomic analyses of *Leuconostoc mesenteroides* provide insights into its genomic and metabolic features and roles in kimchi fermentation. *Scientific Reports*, *7*(1), 11504. https://doi.org/10.1038/s41598-017-12016-z

Cynober, L. (2018). Metabolism of dietary glutamate in adults. *Annals of Nutrition and Metabolism*, *73*(Suppl 5), 5–14. https://doi.org/10.1159/000494776

Egbujor, M. C., Olaniyan, O. T., Emeruwa, C. N., Saha, S., Saso, L., & Tucci, P. (2024). An insight into the role of amino acids as antioxidants via NRF2 activation. *Amino Acids*, *56*(1), 23. https://doi.org/10.1007/s00726-024-03384-8

Fachrial, E., Ismawati, Jati, A. P., Nugroho, T. T., & Saryono. (2025). Isolation and characterization of lactic acid bacteria from “trites” having the ability to produce α-glucosidase inhibitors. *International Journal of Microbiology*, *2025*(1), 8864668. https://doi.org/10.1155/ijm/8864668

Ganguly, S. (2023). The pivotal role of *Corynebacterium glutamicum* in l-glutamic acid fermentation: A concise review. *Biocatalysis and Agricultural Biotechnology*, *47*, 102578. https://doi.org/10.1016/j.bcab.2022.102578

Ghazanfari, N., Fallah, S., Vasiee, A., & Yazdi, F. T. (2023). Optimization of fermentation culture medium containing food waste for l-glutamate production using native lactic acid bacteria and comparison with industrial strain. *LWT - Food Science and Technology*, *184*, 114871. https://doi.org/10.1016/j.lwt.2023.114871

Goa, T., Beyene, G., Mekonnen, M., & Gorems, K. (2022). Isolation and characterization of lactic acid bacteria from fermented milk produced in Jimma Town, Southwest Ethiopia, and evaluation of their antimicrobial activity against selected pathogenic bacteria. *International Journal of Food Science*, *2022*(1), 2076021. https://doi.org/10.1155/2022/2076021

Gökmen, G. G., Sarıyıldız, S., Cholakov, R., Nalbantsoy, A., Baler, B., Aslan, E., Düzel, A., Sargın, S., Göksungur, Y., & Kışla, D. (2024). A novel *Lactiplantibacillus plantarum* strain: Probiotic properties and optimization of the growth conditions by response surface methodology. *World Journal of Microbiology and Biotechnology*, *40*, 66. https://doi.org/10.1007/s11274-023-03862-3

Gonzalez, J. M., & Aranda, B. (2023). Microbial growth under limiting conditions-future perspectives. *Microorganisms*, *11*(7), 1641. https://doi.org/10.3390/microorganisms11071641

Guadarrama-Pérez, O., Moeller-Chávez, G. E., Bustos-Terrones, V., Guillén-Garcés, R. A., Hernández-Romano, J., Barragán-Trinidad, M., Estrada-Arriaga, E. B., & Guadarrama-Pérez, V. H. (2024). Identification of sugars as root exudates of the macrophyte species *Juncus effusus* and *Philodendron cordatum* in constructed wetland-microbial fuel cells during bioelectricity production. *Environmental Technology*, *45*(4), 716-730. https://doi.org/10.1080/09593330.2022.2121180

Guryanov, I., & Naumenko, E. (2024). Bacterial Pigment prodigiosin as multifaceted compound for medical and industrial application. *Applied Microbiology*, *4*(4), 1702-1728. https://doi.org/10.3390/applmicrobiol4040115

Gwin, J. A., Church, D. D., Wolfe, R. R., Ferrando, A. A., & Pasiakos, S. M. (2020). Muscle protein synthesis and whole-body protein turnover responses to ingesting essential amino acids, intact protein, and protein-containing mixed meals with considerations for energy deficit. *Nutrients*, *12*(8), 2457. https://doi.org/10.3390/nu12082457

Helmstetter, F., Arnold, P., Höger, B., Petersen, L. M., & Beitz, E. (2019). Formate–nitrite transporters carrying nonprotonatable amide amino acids instead of a central histidine maintain pH-dependent transport. *Journal of Biological Chemistry*, *294*(2), 623–631. https://doi.org/10.1074/jbc.RA118.006340

Hussein, M. A., Hameed, R. M., & Hnewa, R. A. A. (2021). Qualitative analysis of standard amino acid by thin layer chromatography in examination of inborn errors of metabolism. *Scientific Journal of Medical Research*, 5(19), 73-77.

Ismail, Y. S., Yulvizar, C., & Putriani. (2017). Isolasi, karakterisasi dan uji aktivitas antimikroba bakteri asam laktat dari fermentasi biji kakao (*Theobroma cacao* L.). *Bioleuser*, *1*(2), 45–53.

Kamble, C., Chavan, R., & Kamble, V. (2021). A review on amino acids. *Research & Reviews: A Journal of Drug Design & Discovery*, *8*(3), 19–27. https://doi.org/10.37591/RRJoDDD

Lee, J., Heo, S., Choi, J., Kim, M., Pyo, E., Lee, M., Shin, S., Lee, J., Sim, J., & Jeong, D. W. (2021). Selection of *Lactococcus lactis* HY7803 for glutamic acid production based on comparative genomic analysis. *Journal of Microbiology and Biotechnology*, *31*(2), 298–303. https://doi.org/10.4014/jmb.2011.11022

Lemoine, F., & Gascuel, O. (2024). The bayesian phylogenetic bootstrap and its application to short trees and branches. *Molecular biology and evolution*, *41*(11), msae238. https://doi.org/10.1093/molbev/msae238

Liu, H., & Chen, Y. (2018). Enhanced methane production from food waste using cysteine to increase biotransformation of L-monosaccharide, volatile fatty acids, and biohydrogen. *Environmental Science & Technology*, *52*(6), 3777–3785. https://doi.org/10.1021/acs.est.7b05355

Liu, H., Xu, H., Ma, C., Zhu, Z., Xu, T., Guo, Y., & Ye, J. (2022). Review on the intermediate amino acids and their enantiomers during the anaerobic digestion: the distribution, biofunctions, and mechanisms. *Reviews in Environmental Science and Bio/Technology*, *21*(2), 469–482. https://doi.org/10.1007/s11157-022-09614-3

Mahdi, M. J., & Hidayat, R. (2022). Prarancangan pabrik monosodium glutamat dari molase dan urea dengan proses biosintesis kapasitas 80.000 ton/tahun. *Jurnal Tugas Akhir Teknik Kimia*, *5*(1), 65–69.

Maldonado-Ruiz, K., Pedroza-Islas, R., & Pedraza-Segura, L. (2024). Blue biotechnology: marine bacteria bioproducts. *Microorganisms*, *12*(4), 697. https://doi.org/10.3390/microorganisms12040697

Mardiana, N. A., Murniasih, T., Rukmi, W. D., & Kusnadi, J. (2020). Potensi bakteri laut sebagai sumber antibiotik baru penghambat *Staphylococcus aureus*. *Jurnal Teknologi Pertanian*, *21*(1), 49–56. https://doi.org/10.21776/ub.jtp.2020.021.01.6

Maslami, V., Marlida, Y., Mirnawati, & Nur, Y. S. (2023). The potency of glutamate producing lactic acid bacteria isolated as additive feed in improving broiler performance. *Jurnal Ilmu dan Teknologi Peternakan Tropis*, 10(1), 62–68.

Maslami, V., Marlida, Y., Mirnawati, M., Jamsari, J., & Nur, Y. S. (2018). Isolasi bakteri asam laktat (BAL) penghasil asam glutamat dari ikan budu sebagai *feed* suplemen ayam broiler. *Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science)*, *20*(1), 29−36. https://doi.org/10.25077/jpi.20.1.29-36.2018

Melia, S., Juliyarsi, I., Kurnia, Y. F., Pratama, Y. E., & Pratama, D. R. (2020). The quality of fermented goat milk produced by *Pediococcus acidilactici* BK01 on refrigerator temperature. *Biodiversitas*, *21*(10), 4591–4596. https://doi.org/10.13057/biodiv/d211017

Ojha, K. K., Mishra, S., & Singh, V. K. (2022). Computational molecular phylogeny: Concepts and applications. *Bioinformatics* (pp. 67–89). Elsevier. https://doi.org/10.1016/B978-0-323-89775-4.00025-0

Pangestika, W., Al-Baarri, A. N. M., & Legowo, A. M. (2018). The change in pH on *Lactobacillus acidophilus* medium containing D-fructose. *Journal of Applied Food Technology*, 5(2), 37–38. https://doi.org/10.17728/jaft.4870

Papadimitriou, K., Alegría, Á., Bron, P. A., De Angelis, M., Gobbetti, M., Kleerebezem, M., Lemos, J. A., Linares, D. M., Ross, P., Stanton, C., Turroni, F., van Sinderen, D., Varmanen, P., Ventura, M., Zúñiga, M., Tsakalidou, E., & Kok, J. (2016). Stress physiology of lactic acid bacteria. *Microbiology and Molecular Biology Reviews*, *80*(3), 837–890. https://doi.org/10.1128/MMBR.00076-15

Quintanilla-Villanueva, G. E., Rodríguez-Delgado, M. M., Villarreal-Chiu, J. F., Blanco-Gámez, E. A., & Luna-Moreno, D. (2024). The role of amino acid glycine on cardiovascular health and its beneficial effects: A narrative review. *Journal of Vascular Diseases*, *3*(2), 201–211. https://doi.org/10.3390/jvd3020016

Rahmayetty, R., Yulvianti, M., & Hartono, R. (2022). Synthesis of lactic acid from molasses by Lactobacillus acidophilus using a batch fermentation process. *Jurnal Rekayasa Kimia dan Lingkungan*, *17*(2), 104–113. https://doi.org/10.23955/rkl.v17i2.25657

Ramesha, V., & Deeksha, R. N. (2019). Production of secondary metabolites. *The Pharma Innovation Journal*, *8*(12), 46–48.

Sasmita, S., Halim, A., Sapriati, A. N., & Kursia, S. (2018). Isolasi dan identifikasi bakteri asam laktat dari liur basa (limbah sayur bayam dan sawi). *As-Syifaa*, *10*(2), 141–151. https://doi.org/10.33096/jifa.v10i2.339

Shangguan, L., Zhang, H., Liu, Z., An, F., Yang, Q., Zhang, X., Yao, L., Yang, S., Dai, J., & Chen, X. (2023). Improved glutamic acid production capacity of *Corynebacterium glutamicum* by the ARTP mutagenesis method. *Fermentation*, *9*(7), 599. https://doi.org/10.3390/fermentation9070599

Sheikh, H., Anand, G. G., & Shivanna, G. B. (2024). Fermentation: a potential strategy for microbial metabolite production. *IntechOpen*. https://doi.org/10.5772/intechopen.114814

Shehata, M. G., El Sohaimy, S. A., El-Sahn, M. A., & Youssef, M. M. (2016). Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. *Annals of Agricultural Sciences*, *61*(1), 65–75. https://doi.org/10.1016/j.aoas.2016.03.001

Song, L., Yang, D., Liu, R., Liu, S., & Dai, X. (2023). The dissolution of polysaccharides and amino acids enhanced lactic acid production from household food waste during pretreatment process. *Science of the Total Environment*, *864*, 161068. https://doi.org/10.1016/j.scitotenv.2022.161068

Syahputra, A., Mutaqin, K. H., & Damayanti, T. A. (2016). Komparasi metode isolasi DNA patogen antraknosa dan bulai untuk deteksi PCR. *Jurnal Fitopatologi Indonesia*, *12*(4), 124–124.

Tathode, M. S., Bonomo, M. G., Zappavigna, S., Mang, S. M., Bocchetti, M., Camele, I., Caraglia, M., & Salzano, G. (2024). Whole-genome analysis suggesting probiotic potential and safety properties of *Pediococcus pentosaceus* DSPZPP1, a promising LAB strain isolated from traditional fermented sausages of the Basilicata region (Southern Italy). *Frontiers in Microbiology*, *15*, 1268216. https://doi.org/10.3389/fmicb.2024.1268216

Thuy, L. N., Salanță, L., Tofană, M., Socaci, S. A., Fărcaș, A. C., & Pop, C. R. (2020). A mini review about monosodium glutamate. *Bulletin UASVM Food Science and Technology*, *77*(1), 1–12. https://doi.org/10.15835/buasvmcn-fst:2019.0029

Toe, C. J., Foo, H. L., Loh, T. C., Mohamad, R., Rahim, R. A., & Idrus, Z. (2019). Extracellular proteolytic activity and amino acid production by lactic acid bacteria isolated from Malaysian foods. *International Journal of Molecular Sciences*, *20*(7), 1777. https://doi.org/10.3390/ijms20071777

Tunio, A. A., Naqvi, S. H., Tunio, Q., Rehman, T., Bhutto, M. A., & Mugheri, M. H. (2022). Determination of antioxidant, antimicrobial properties with evaluation of biochemicals and phytochemicals present in *Oscillatoria limosa* of District Jamshoro, Pakistan. *Yuzuncu Yıl University Journal of Agricultural Sciences*, *32*(3), 538–547. https://doi.org/10.29133/yyutbd.1112896

Wang, M., Zhang, X., Huang, H., Qin, Z., Liu, C., & Chen, Y. (2022). Amino acid configuration affects volatile fatty acid production during proteinaceous waste valorization: Chemotaxis, quorum sensing, and metabolism. *Environmental Science & Technology*, *56*(12), 8702–8711. https://doi.org/10.1021/acs.est.1c07894

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology*, *9*, 612285. https://doi.org/10.3389/fbioe.2021.612285

Wang, Z., Lao, J., Kang, X., Xie, Z., He, W., Liu, X., Zhong, C., Zhang, S., & Jin, J. (2023). Insights into the metabolic profiling of Polygonati Rhizoma fermented by *Lactiplantibacillus plantarum* under aerobic and anaerobic conditions using a UHPLC-QE-MS/MS system. *Frontiers in Nutrition*, *10*, 1093761. https://doi.org/10.3389/fnut.2023.1093761

Yeo, K., Li, R., Wu, F., Bouras, G., Mai, L. T. H., Smith, E., Wormald, P., Valentine, R., Psaltis, A. J., Vreugde, S., & Fenix, K. (2024). Identification of consensus head and neck cancer-associated microbiota signatures: A systematic review and meta-analysis of 16S rRNA and The Cancer Microbiome Atlas datasets. *Journal of Medical Microbiology*, *73*(2), 001799. https://doi.org/10.1099/jmm.0.001799

Zaghloul, E. H., & Halfawy, N. M. E. (2024). Marine *Pediococcus pentosaceus* E3 probiotic properties, whole-genome sequence analysis, and safety assessment. *Probiotics and Antimicrobial Proteins*, *16*, 1925–1936. https://doi.org/10.1007/s12602-024-10283-7

Zhang, H., Wang, Z., Li, Z., Wang, K., Kong, B., & Chen, Q. (2022). L-glycine and L-glutamic acid protect *Pediococcus pentosaceus* R1 against oxidative damage induced by hydrogen peroxide. *Food Microbiology*, *101*, 103897. https://doi.org/10.1016/j.fm.2021.103897

Zhang, Y., Venkitasamy, C., Pan, Z., & Liu, W. (2017). Novel umami ingredients: Umami peptides and their taste. *Journal of Food Science*, *82*(1), 16–23. https://doi.org/10.1111/1750-3841.13576

Zhao, J., Siddiqui, S., Shang, S., Bian, Y., Bagchi, S., He, Y., & Wang, C. R. (2015). Mycolic acid-specific T cells protect against *Mycobacterium tuberculosis* infection in a humanized transgenic mouse model. *eLife*, *4*, e08525. https://doi.org/10.7554/eLife.08525

Zhou, Y., Zhang, X., Wang, Y., & Liu, H. (2024). Mechanism and effect of amino acids on lactic acid production in acidic fermentation of food waste. *Fermentation*, *10*(4), 179. https://doi.org/10.3390/fermentation10040179