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ORIGINAL RESEARCH PAPER

Characterization and comparative genomic analysis of gamma-aminobutyric acid (GABA)-producing lactic acid bacteria from Thai fermented foods

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Abstract

Objectives This study aimed to screen, characterize, and annotate the genome along with the comparison of GABA synthesis genes presented in lactic acid bacteria (LAB).

Results Thirty-five LAB isolates from fermented foods were screened for GABA production using thin-layer chromatography (TLC). Fifteen isolates produced GABA ranging from 0.07 to 22.94 g/L. Based on their GTG_5 profiles, phenotypic, and genotypic characteristics, isolates LSI1-1, LSI1-5, LSI2-1, LSI2-2, LSI2-3, LSI2-5, and LSM3-1-4 were identified as *Lactobacillus plantarum* subsp. *plantarum*; isolate LSM1-4 was *Lactobacillus argentoratensis*; isolates CAB1-2, CAB1-5, CAB1-7, and LSI1-4 were *Lactobacillus pentosus*; and CAB1-1, LSM3-1-1 and LSM3-2-3 were *Lactobacillus fermentum*. Strains LSI2-1 and CAB1-7 from pickled vegetables were selected for genome analysis. The *gadA* gene (1410 bp, 470aa) was encountered in GABA production of both strains and no other glutamate decarboxylase (GAD) genes were found in the genomes when compared with other LAB strains. The presence of *gadA* is evidence for GABA production. Strains LSI2-1 and CAB1-7 produced 22.94 g/L and 11.59 g/L of GABA in GYP broth supplemented with 3% (w/v) MSG at 30 °C for 72 h, respectively.

Conclusions Our report highlights the characterization of LAB and GABA production of *L. plantarum* LSI2-1 strain with its GABA synthesis gene.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10529-021-03140-y.

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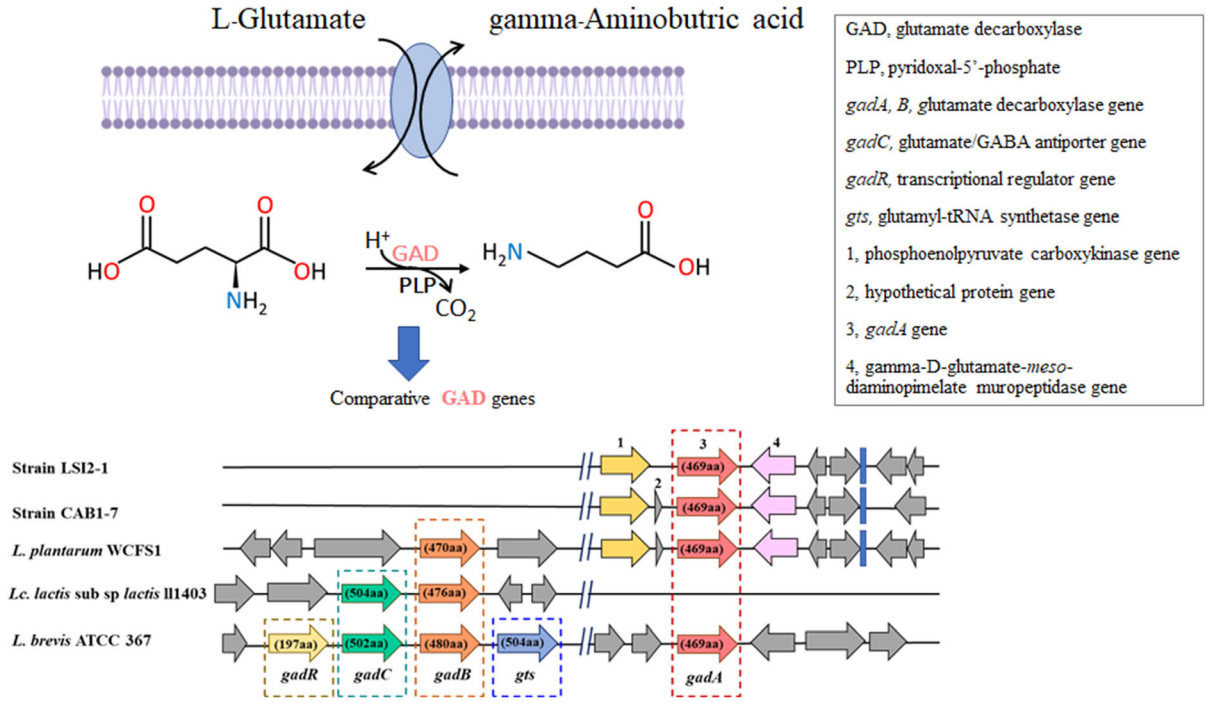
Graphic abstract GABA production of strains LSI2-1 and CAB1-7 in GYP broth with 3% (w/v) MSG and comparative GAD genes



Keywords Gamma-aminobutyric acid (GABA) · Genome analysis · Glutamate decarboxylase (GAD) · Lactic acid bacteria (LAB) · *Lactobacillus plantarum*

Introduction

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the sympathetic nervous system (Mody et al. 1994) for controlling stress (Oh and Oh 2003), preventing Alzheimer's disease (Nakashima et al. 2009), and reducing depression (Okada et al. 2000). It also plays a role in cardiovascular function (De Feudis 1983) and ameliorates diabetic conditions (Hagiwara et al. 2004). In addition, GABA has positive benefits for human health including treatment for diuretic and convulsive effects (Wong et al. 2003), sleeplessness (Okada et al. 2000) and chronic alcohol-related symptoms, while stimulating the immune system and hormone production (Oh and Oh 2003). GABA is synthesized from glutamate decarboxylase (GAD; EC 4.1.1.15), with pyridoxal 5′ phosphate (PLP) as a coenzyme that catalyzes the irreversible decarboxylation of L-glutamate into GABA (Ueno 2000). The GAD enzyme encoded by *gadA* or *gadB* is responsible for the synthesis of GABA in microbial cells, and the decarboxylated product is exported from the cell by an antiporter (Small and Waterman 1998).

Lactic acid bacteria (LAB) are used to produce GABA because they are considered safe and exhibit several health-promoting properties (Nejati et al. 2013). Strains of *Lactobacillus brevis* from goat cheese (Li et al. 2013), *L. senmaizukei* from traditional pickles (Hiraga et al. 2008), *L. plantalum* from Malaysian fermented foods (Zareian et al. 2015), *L. rhamnosus* from Chinese traditional food (Lin 2013), *L. paracasei* from Italian cheese and Japanese fermented fish (Komatsuzaki et al. 2005), and *L. namurensis* from Nham (Thai fermented pork sausage) (Ratanaburee et al. 2013) were all reported to produce GABA.

Different GABA production mechanisms can be explained by genome data of the cellular physiology of each bacterial species. Most reports on GABA

production involved *L. brevis* strains, with little known about the genomes of *L. pantarum* and *L. pentosus* strains. Therefore, this study focused on screening, characterization, and gene description by comparing genome sequences of GABA-producing strains and the GAD systems of related *Lactobacillus* strains.

Materials and methods

Isolation of lactic acid bacteria (LAB)

Six Thai fermented food samples were collected from local markets including pickled *Brassica oleracea* from Bangkok, pickled *Lasia spinosa* (L.) Thwaites (Phak-nam dong) from Singburi Province, pickled *Crateva adansonii* subsp. *trifoliata* Roxb. (Phak-koom-dong) from Singburi Province, and fermented pork and pickled *Allium ascalonicum* (Hom-dong) from Samut Prakarn Province, Thailand (Table 1). Two hundred microliters or 1 g of sample were enriched in 5 mL of Man Rogosa Sharpe (MRS) broth (Difco) and incubated at 30 °C for 24 h. The culture broth was streaked on MRS agar plates with 0.3% (w/v) $CaCO_3$ and cultivated at 30 °C for 24 h. Isolates that exhibited a clear zone were selected to purify on MRS with 0.3% (w/v) $CaCO_3$ agar plates. The selected isolates were preserved in a freezer and by lyophilization until required for further study.

Screening and determination of GABA production

Active cultures were prepared by transfer from glycerol stock into MRS broth and incubated at 30 °C for 24 h. Glucose-yeast extract-peptone (GYP) broth supplemented with 3% (w/v) monosodium glutamate (MSG) (Lyu et al. 2018) was used as the production medium. A culture of each strain (10% v/v inoculum, cultivated at the same condition for 18 h) was inoculated in production medium and cultivated at 30 °C for 72 h. The culture broth was centrifuged at 10,000 rpm for 5 min and GABA production was analyzed qualitatively using thin-layer chromatography (TLC). The supernatant 2 µL was spotted onto TLC plates (aluminum TLC plates silica gel 60 F254 plates, Merck). The plates were developed in a mobile phase including acetic acid: *n*-butanol: distilled water (5:3:2) and subsequently sprayed with 0.4% (w/v) ninhydrin dissolved in ethanol. The plates were heated for several minutes until GABA spots appeared (Villegas et al. 2016).

GABA concentration was determined quantitatively by high-performance liquid chromatography (HPLC) following Silva et al. (2009) with slight modifications. Briefly, 400 µL of sample and 80 µL of 5 mg/mL ortho-phthaldialdehyde were dissolved in absolute methanol, 300 µL of 0.1 M borate buffer and 20 µL mercaptopropionic acid. The solution was mixed immediately, and left to stand at room temperature for 1 min before injection into the equipment as described by Kutlan and Molnar-Perl (2003). The HPLC instrument consisted of a Varian Prostar (Granite Quarry, NC, USA) with a 3 µm particle size (150 mm × 4.6 mm, ID), Luna 5U C18(2) column (Hibar-Futigsanle RT). The experiment was conducted at 25 °C and eluted with a mobile phase system of 85% (v/v) solution A [0.05 M sodium acetate, tetrahydrofuran, and methanol (50:1:49, v/v/v)] and 15% (v/v) solution B (methanol) at a flow rate of 1 mL/min. A fluorescence detector model 363 was set at an excitation wavelength of 337 nm and an emission wavelength of 454 nm.

Phenotypic characteristics

Morphological and cultural characteristics including Gram reaction, cell morphology, and colonial appearance of the isolates were determined after culturing the cells on MRS agar at 30 °C for 24 h. Physiological and biochemical characteristics consisted of growth at different parameters of pH (3, 6, and 9), temperatures (15, 30, and 45 °C), and NaCl concentrations (2, 4, 6, and 8% w/v). Catalase activity, nitrate reduction, gas production, arginine hydrolysis, and acid production from carbohydrates were performed as previously described (Tanasupawat et al. 1998).

Genotypic characteristics

The 16S rRNA gene sequences of isolates were PCR amplified as described by Phuengjayaem et al. (2017). Amplified 16S rRNA gene sequences were sequenced on a DNA sequencer (Macrogen, Korea) using universal primers as described earlier (Lane 1991). Sequence similarity values between the isolates and related reference strains were evaluated on the EzBiocloud server (Yoon et al. 2017). A phylogenetic tree based on the neighbor-joining (NJ) method

Table 1 Phenotypic characteristics and isolation sources of isolates

Characteristics/Isolate no.	Group I *L. plantarum* subsp. *plantarum*							Group II *L. argentoratensis*	Group III *L. pentosus*				Group IV *L. fermentum*		
	LIS1-1	LSI1-5	LSI2-1	LSI2-2	LSI2-3	LSI2-5	LSM3-1-4	LSM1-4	LSI1-4	CAB1-2	CAB1-5	CAB1-7	CAB1-1	LSM3-1-1	LSM3-2-3
Gas from glucose	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Arginine hydrolysis	−	+	+	−	w	−	−	−	+	+	+	+	w	−	−
Nitrate reductase	−	−	+	+	−	−	−	−	−	−	+	−	+	+	+
Growth at pH 3	w	+	+	+	+	+	+	w	+	w	+	w	+	+	+
Growth at 45 °C	w	−	w	−	w	−	w	+	w	w	+	−	+	+	w
Growth in 2% (w/v) NaCl	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Acid From:															
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Melibiose	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	−	+	+	+	+	−	−	+	+	+	+	+	+	+	−
Rhamnose	−	+	+	−	+	−	−	+	+	+	+	+	+	+	−
Salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Sorbitol	−	+	+	−	+	−	−	+	+	+	+	+	+	+	+
Xylose	w	+	+	w	+	−	−	+	+	+	+	+	+	+	+
Isolation source	2	2	3	3	3	3	5	4	2	1	1	1	1	5	5

[1]Pickled *Brassica oleracea* from Bangkok

[2]Pickled *Lasia spinosa* (L.) Thwaites (Phak-nam dong) from Singburi Province

[3]Pickled *Crateva adansonii* subsp. *trifoliata* (Roxb.) (Phak-koom-dong) from Singburi Province

[4]Fermented pork from Samut Prakarn Province

[5]Pickled *Allium ascalonicum* (Hom-dong) from Samut Prakarn Province. + positive reaction; *w* weakly positive reaction; − negative reaction

(Saitou and Nei 1987) and GAD genes tree based on the Maximum-Likelihood were constructed using MEGA 7 (Kumar et al. 2016). Bootstrap analysis was performed to determine the confidence values of individual branches in the phylogenetic tree with 1000 replications (Felsenstein 1985). Identified sequences were deposited in DDBJ (DNA Data Bank of Japan, Mishima, Japan).

Crude DNA of isolates was extracted from cell pellets using the alkaline-polyethylene glycol-based method (Alkaline-PEG) (Chomczynski and Rymaszewski 2006). A DNA fingerprint was also performed based on the $(GTG)_5$-PCR method. PCR amplification, gel electrophoresis, and analysis methods were conducted as previously described (Tolieng et al. 2018). *L. plantarum* subsp. *plantarum* NRIC 1067^T and *L. pentosus* NRIC 1069^T were used as reference strains.

Genome sequencing and assembly

Genomic DNA of representative strains was extracted by a phenol–chloroform method and purified according to the procedure of Tamaoka (1994). The genomes of each strain were sequenced with an Illumina Miseq platform (Illumina, Inc., San Diego, US-CA) using 2×250 bp paired-end reads assembled to contig using SPAdes 3.12 (Bankevich et al. 2012). Comparative genomic analyses were annotated using the RAST server (Aziz et al. 2008) in accordance with the NCBI prokaryotic genome annotation pipeline (PGAP) and performed for comparison using the SEED Viewer (Aziz et al. 2012). Draft genomes of the strains were publicly available from GenBank, and nucleotide data of closely related strains were obtained from the GenBank database.

Statistical analysis

All experiments were performed in triplicate, with data presented as mean ± standard deviation (SD). The statistical package for the social sciences (SPSS) for Windows program version 15 was used for data analysis, with one-way ANOVA utilized for comparison of each factor and Tukey's multiple range test for pairwise comparison at p-value of 0.05.

Results and discussion

Isolation, screening, and determination of GABA production

Thirty-five isolates from fermented food were qualitatively screened for GABA production using the TLC method. Fifteen isolates including CAB1-1, CAB1-2, CAB1-5, CAB1-7, LSI1-1, LSI1-4, LSI1-5, LSI2-1, LSI2-2, LSI2-3, LSI2-5, LSM1-4, LSM3-1-1, LSM3-1-4, and LSM3-2-3 exhibited positive spots at R_f 0.57 compared with the spot of GABA standard (10 g/L). All isolates were quantitatively determined for GABA production using HPLC. The GABA standard curves showed a linear relationship ($R^2 = 0.991$) between peak areas and GABA concentrations ranging from 2 to 16 mg/L (Supplementary Fig. S1). MSG and GABA chromatogram of HPLC are presented in Supplementary Fig. S2. Based on the statistical analysis results, different capabilities of GABA production by the 15 isolates were divided into five groups (Supplementary Tables S1 and S2). In the first group, isolates LSI2-1 and LSI2-3, exhibited GABA production of 22.94 and 17.45 g/L, respectively (Fig. 1). An increase in GABA production resulted in a decrease of MSG concentration. The second group, LSI2-3, CAB1-7, LSI1-1, LSI2-2, and LSI2-5 gave 17.45, 11.59, 10.77, 10.43, and 7.89 g/L of GABA, respectively. The remaining eight isolates produced GABA ranging from 0.07 to 7.22 g/L with high remaining MSG (Fig. 1). From previous reports, *L. brevis* strains IFO-12005 (Yokoyama et al. 2002) and HYE1 (Lim et al. 2017) produced GABA at 1.05 and 2.21 g/L respectively, while *L. rhamnosus* strain GG (Song and Yu 2018) and YS9 (Lin 2013), *L. fermentum* W1 (Rayavarapu et al. 2019), *L. plantarum* Taj-Apis 362 (Tajabadi et al. 2015) and *Lactococcus lactis* B (Lu et al. 2009) produced 1.13, 19.28, 5.34, 0.74, and 7.20 g/L of GABA, respectively. Here, strains LSI2-1, LSI2-3 displayed beneficial GABA production with no significant difference in quantity. Multiple comparison analysis using Tukey's method at α 0.05 showed that isolates LSI2-1 and LSI2-3 (p-value 0.749) were classified in the same group (Supplementary Tables S1 and S2). Isolate LSI2-1 produced maximum GABA concentration at a higher efficiency rate.

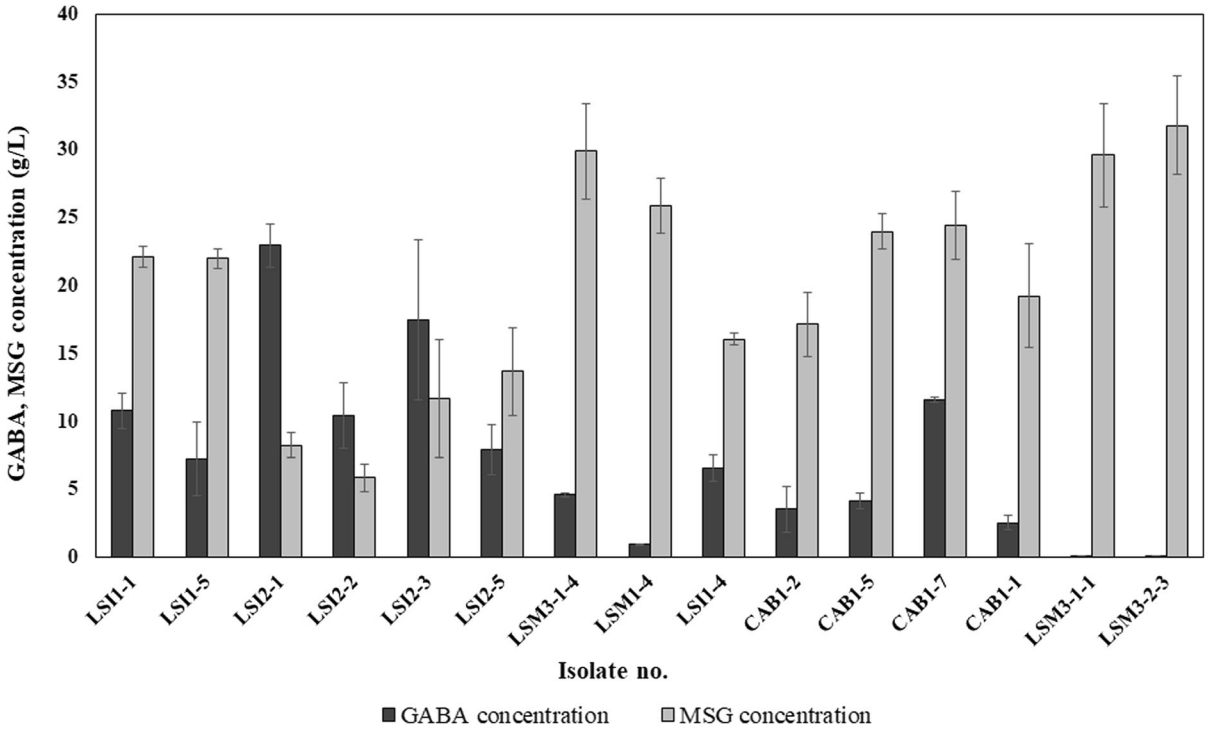


Fig. 1 GABA production and residual MSG (g/L) of isolates cultivated in GYP with 3% (w/v) MSG, at 30 °C for 3 days

Identification of isolates

All isolates consisted of catalase-negative rod-shaped bacteria and formed milky-white, circular, convex, and opaque colonies. The isolates produced acid from arabinose, fructose, glucose, maltose, ribose, sucrose, and trehalose with growth at 15–37 °C, pH 3–9 and 4–8% (w/v) NaCl. Their phenotypic characteristics are listed in Table 1.

The $(GTG)_5$-PCR analysis was used to detect intraspecific variability of the strains. The number of detectable amplicons varied from 10 to 20, with DNA band size ranging from 300 to 10,000 bp. The UPGMA dendrogram relied on DNA fingerprinting using the $(GTG)_5$ primer and was clustered into five groups (Fig. 2). Three isolates CAB1-2, CAB1-5, and CAB1-7 were clustered with *L. pentosus* NRIC 1069^T, while isolates LSI2-1, LSI2-2, and LSI2-3 were clustered in the same group, and LSI1-1, LSI1-5 and LSI2-5 were clustered with *L. plantarum* NRIC 1067^T. The profile of CAB1-1 was the same as LSM3-1-1 and LSM3-2-3, while isolates LSI1-4, LSM1-4, and LSM3-1-4 exhibited unique profiles.

On the basis of their phenotypic characteristics (Table 1), DNA fingerprinting pattern (Fig. 2), and the results of 16S rRNA gene sequences (Fig. 3) the isolates were divided into four groups as described below.

Group I consisted of seven isolates including LSI1-1, LSI1-5, LSI2-1, LSI2-2, LSI2-3, LSI2-5, and LSM3-1-4. Strains LSI1-1 (1385 bp), LSI1-5 (1428 bp), LSI2-1 (1419 bp), LSI2-2 (1410 bp), and LSM3-1-4 (1401 bp) were closely related to *L. plantarum* subsp. *plantarum* ATCC 14917^T with 100% sequence similarity, while LSI2-3 (1441 bp) and LSI2-5 (1412 bp) were also closely related to *L. plantarum* subsp. *plantarum* ATCC 14917^T (Bringel et al. 2005) with 99.93 and 99.72% sequence similarity, respectively (Fig. 3). Therefore, they were identified as *Lactobacillus plantarum* subsp. *plantarum*.

Group II contained only the strain LSM1-4 (1361 bp) that was closely related to *L. argentoratensis* DSM 16365^T (Bringel et al. 2005) with 99.93% sequence similarity. *L. argentoratensis* DSM 16365^T was previously allocated to *Lactobacillus plantarum* subsp. *argentoratensis* (Bringel et al. 2005). In this study, strain LSM1-4 exhibited a 16S rRNA gene sequence

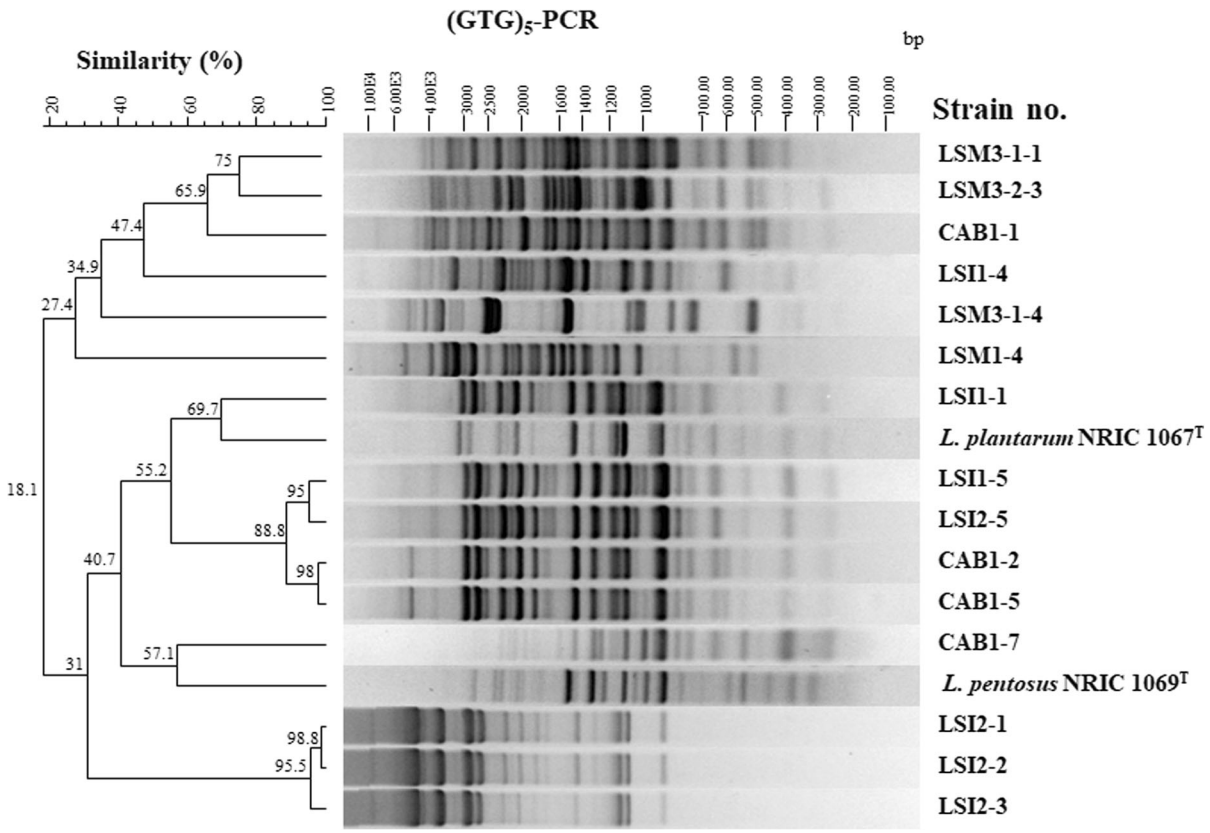


Fig. 2 $(GTG)_5$-PCR banding patterns of GABA-producing strains. The dendrogram was generated after cluster analysis of digitized fingerprints and derived from the UPGMA linkage of Pearson correlation coefficients

(Fig. 3) similar to strains in Group I but showed independent difference in DNA fingerprinting $(GTG)_5$ (Fig. 2) from Group I strains. Therefore, it was identified as *Lactobacillus argentoratensis*.

The 16S rRNA gene sequence of isolates LSI1-1, LSI1-5, LSI2-1, LSI2-2, LSI2-3, LSI2-5, LSM3-1-4, and LSM1-4 were deposited in the DDBJ Database as accession numbers LC616682, LC616683, LC546823, LC546824, LC546825, LC546826, LC616687, and LC620497, respectively.

Group III consisted of four isolates including CAB1-2, CAB1-5, CAB1-7, and LSI1-4. Strains CAB1-2 (1253 bp), CAB1-5 (1337 bp), CAB1-7 (1415 bp), and LSI1-4 (1358 bp) were closely related to *L. pentosus* DSM 20314^T (Zanoni et al. 1987) with 99.52, 100, 99.58, and 99.63% sequence similarity, respectively (Fig. 3). Therefore, they were identified as *Lactobacillus pentosus*. The 16S rRNA gene sequences of isolates CAB1-2, CAB1-5, CAB1-7, and LSI1-4 were deposited in the DNA DDBJ Database under accession numbers LC616686, LC546821, LC546822, and LC616684, respectively.

Group IV contained three isolates including CAB1-1, LSM3-1-1, and LSM3-2-3. Strains CAB1-1 (1429 bp), LSM3-1-1 (1373 bp), and LSM3-2-3 (1432 bp) were closely related to *L. fermentum* CECT 562^T (Beijerinck 1901) with 99.86, 99.71, and 99.93% sequence similarity, respectively (Fig. 3). Therefore, they were identified as *Lactobacillus fermentum*. Sequences of CAB1-1, LSM3-1-1, and LSM3-2-3 were deposited in the DDBJ Database under accession numbers LC616685, LC577832, and LC616688, respectively.

From the results of GABA production, phenotypic characteristics, the $(GTG)_5$-PCR profile, and16S rRNA gene sequences. LSI2-1 was selected as a representative strain of the *L. plantarum* group for genome analysis because it showed maximum efficiency of GABA production, while CAB1-7 was selected as a representative strain of the *L. pentosus*

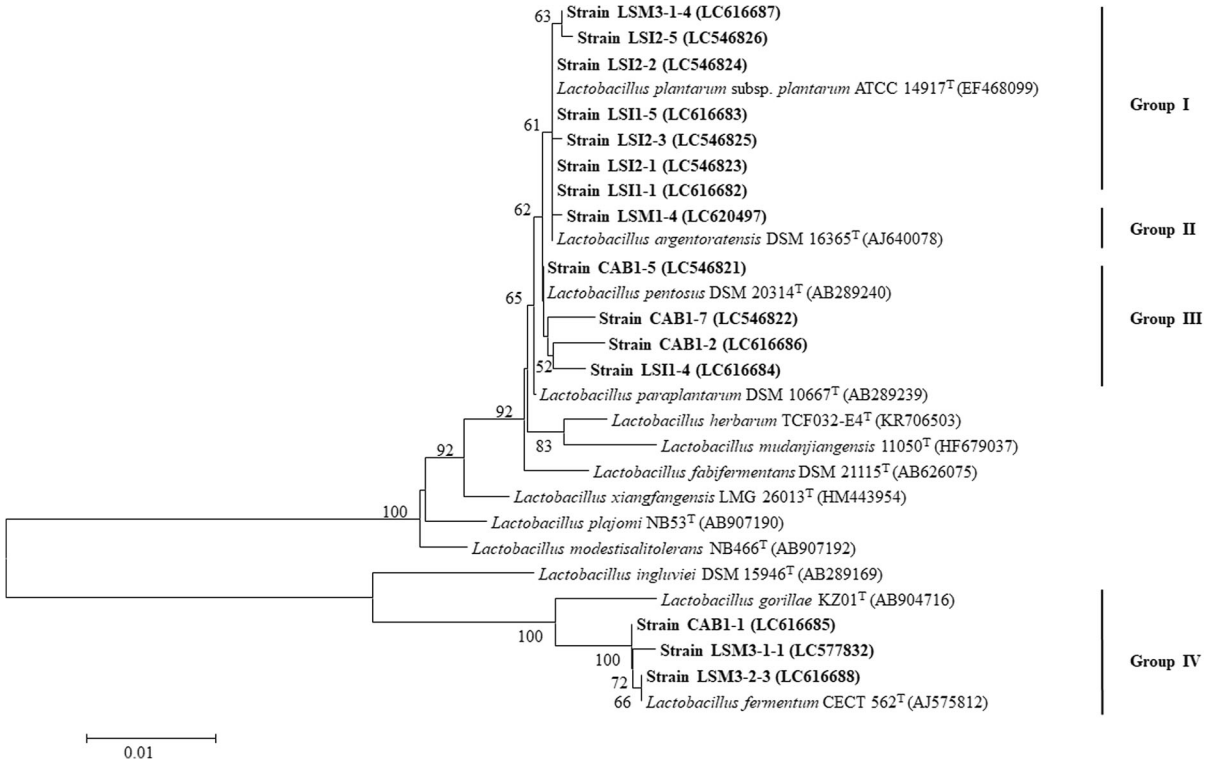


Fig. 3 Neighbor-joining tree based on the 16S rRNA gene sequences showing relationships among the isolates and related species. Numbers on the branches indicate percentage bootstrap values of 1000 replicates, with only values greater than 50% indicated. Bar, 0.01 substitutions per nucleotide position

group to compare the genes based on genome sequencing analysis.

Genome annotation

Draft genome sequences of strains LSI2-1 and CAB1-7 were deposited in GenBank with accession numbers VSFJ00000000 and VSFK00000000, respectively (A genome circular maps are shown in supplementary Fig. S3). Genome features of strain LSI2-1 included 3,222,735 bp with GC content of 44.5 mol%, 32 contigs with protein-encoding genes (PEGs), 336 subsystems, 3087 coding sequences, and 64 RNAs, while the genome features of strain CAB1-7 were 3,201,382 bp with GC content of 44.6 mol%, 74 contigs with PEGs, 332 subsystems, 3055 coding sequences, and 69 RNAs. The *gadA* gene of LSI2-1 (470aa) was coding sequences (CDS) with no subsystem, 1410 bp (676,377–674,968 bp), and *gadA* (470 aa) of strain CAB1-7 was CDS with no subsystem. The size of *gadA* was 1410 bp located from 54,511 to 55,920 bp. The GAD gene of LSI2-1 was closely related to the GAD gene of *L. plantarum* subsp. *plantarum* ATCC 14917^T with 100% similarity while the GAD gene of CAB1-7 was closely related to the GAD gene of *L. plantarum* subsp. *plantarum* ATCC 14917^T with 99.79% similarity.

Comparative genomic analysis

Comparison of the GAD gene between strains LSI2-1, CAB1-7, and other *Lactobacillus* strains of 20 genomic sequencings using a maximum-likelihood tree indicated that both strains were closely related to *L. plantarum* in one branch (Fig. 4a). The *gadA* gene (No. 3 in Fig. 4b) of LSI2-1 and CAB1-7 was located between phosphoenolpyruvate carboxykinase gene (No.1 in Fig. 4b), hypothetical protein gene (No. 2 in Fig. 4b) and gamma-D-glutamate-*meso*-diaminopimelate muropeptidase gene (No.4 in Fig. 4b) the same as *L. plantarum* WCFS1 (Kleerebezem et al. 2003). However, only strain LSI2-1 had no hypothetical

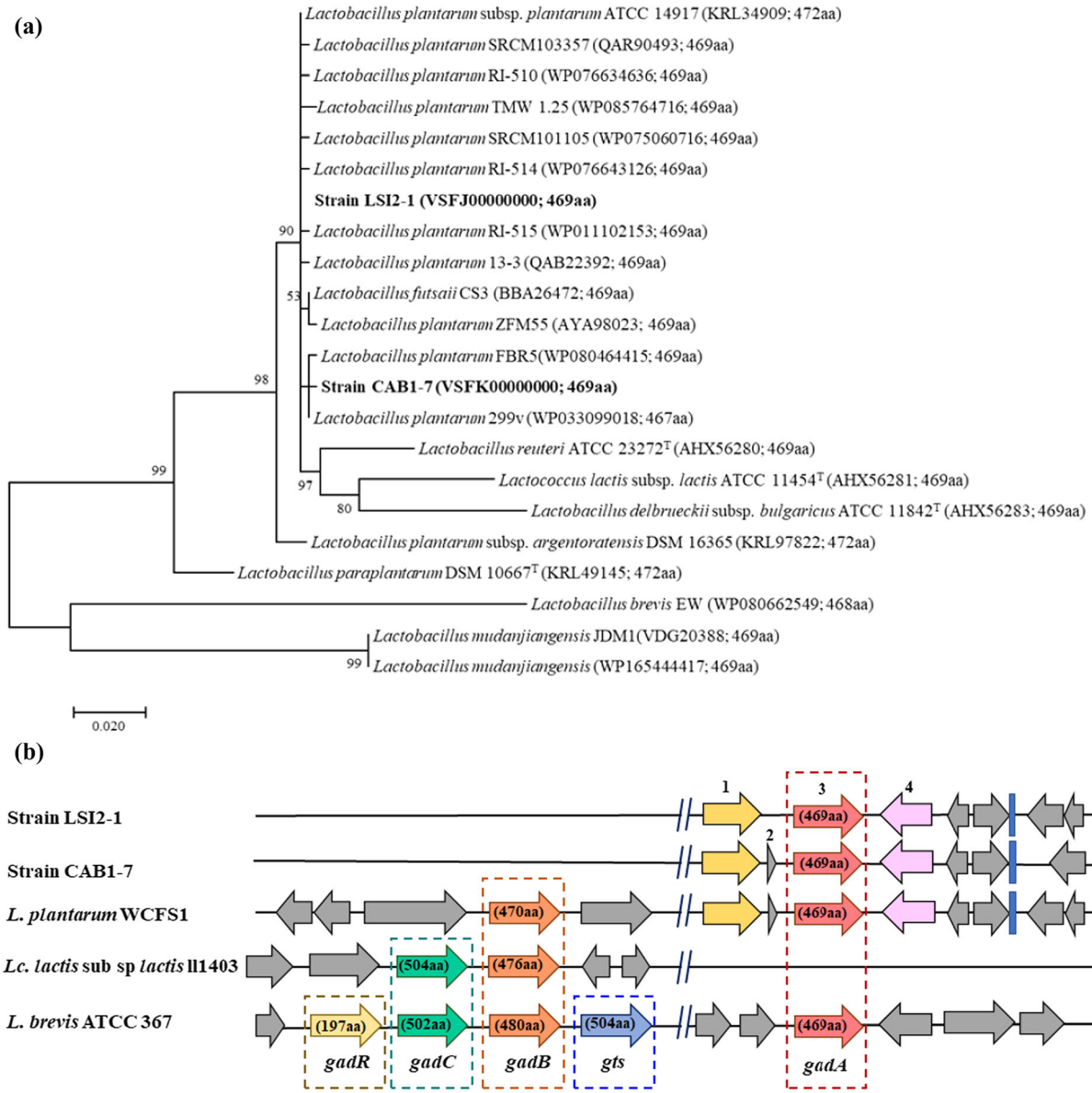


Fig. 4 Comparison of GAD genes in strains CAB1-7 and LSI2-1 and related strains. **a** Maximum-likelihood tree based on amino acid sequences of GAD genes. Numbers on the branches indicate percentage bootstrap values of 1000 replicates. Accession number and length of GAD gene are shown in parentheses. **b** GAD genes of LSI2-1, CAB1-7 and related strains. 1, phosphoenolpyruvate carboxykinase gene; 2, hypothetical protein gene; 3, *gadA* gene; 4, gamma-D-glutamate-*meso*-diaminopimelate muropeptidase gene

protein gene (No. 2 in Fig. 4b). Both *gadA* and *gadB* were found in the GAD gene of *L. brevis* 367^T. The *gadB* gene was located next to the *gadC* gene (Fig. 4b) and the whole operon including *gts* and *gadR,* while *gadA* was located separately from the other *gad* genes (Lyu et al. 2018). The *L. brevis* strain was reported to show potential for GABA production based on *gadA* (Wu et al. 2017) while *Lc. lactis* subsp. *lactis* B produced maximum GABA of 7.2 g/L from 15 g/L MSG at 31.8 °C, pH 7.1 (Lu et al. 2009). By contrast, the *gadA* gene was absent in *Lc. lactis* subsp. *lactis* ll1403, but *gadB* and *gadC* were present. The GAD genes consisted of two isoforms: *gadA* or *gadB*, that exhibited similar functions to convert L-glutamate to

GABA. Moreover, both isoforms of the GAD gene were biochemically indistinguishable (De Biase et al. 1996). Only *gadA* was found in strains LSI2-1and CAB1-7; however, they could produce GABA. Efficiency of GABA production depended on the levels of expression of the GAD gene and other enzymes involved in the glutamate pathway. Histone-like nucleoid-structuring (H-NS) depressed *gad* expression during the log phase, whereas RNA polymerase sigma (*rpoS*) relieved H-NS repression during the stationary phase; directly or indirectly accounting for transcription of GAD genes (De Biase et al. 1999). The *rpoS* gene, which induced *gadA* expression, was found in LSI2-1 and CAB1-7, whereas H-NS was absent. Temperature, pH, medium composition, and other factors also impacted GABA production. Therefore, expression of the *gadA* gene, enzyme activity and production conditions should be determined to enhance GABA production efficiency.

Conclusions

Our phenotypic, genotypic and genome analysis results demonstrated that LAB isolated from fermented food as strains LSI2-1 and CAB1-7 showed capability of GABA biosynthesis at 22.94 g/L and 11.59 g/L, respectively. Strains LSI2-1 and CAB1-7 were identified as *L. plantarum* subsp. *plantalum* and *L. pentosus* based on their 16S rRNA gene sequences. The *gadA* gene was found in both strains as functionally evident GAD-encoding ability. The GABA optimization of strains LSI2-1 and CAB1-7 and their application in fermented food should be investigated in future studies.

Acknowledgements We thank Dr. Nattakorn Kuncharoen for genome sequence analysis, and Mr. Engkarat Kingkaew and Ms. Saranporn Poothong for 16S rRNA gene sequence analysis.

Supplementary Information Supplementary Fig. S1—Calibration curve of GABA concentration in the range of 0–16 µg/mL.

Supplementary Fig. S2—Chromatograms of MSG and GABA analysis using HPLC.

Supplementary Fig. S3—A genome circular maps of strain LSI2-1 (**a**) and CAB1-7 (**b**) created by the 'circular viewer' functionality implemented in the PATRIC web server.

Supplementary Table S1—ANOVA table showed the efficiency on GABA production of isolates.

Supplementary Table S2—The multiple comparisons of GABA production of isolates using Tukey's multiple range tests.

Author contributions SP performed the isolation, screening, identification, genome sequence analysis and writing the manuscript, AB contributed to the GTG_5 profiles, and ST performed the identification and writing the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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