RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2022.037

ScienceAsia 48 (2022): **254–262**

Identification of gamma-aminobutyric acid (GABA)-producing lactic acid bacteria from plant-based Thai fermented foods and genome analysis of *Lactobacillus brevis* GPB7-4

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Received 17 May 2021, Accepted 1 Dec 2021
Available online 28 Feb 2022

ABSTRACT: Sixteen gamma-aminobutyric acid (GABA)-producing lactic acid bacteria (LAB) strains were isolated from plant-based Thai fermented foods, such as fermented bamboo shoots (*naw-mai-dong*), pickled green mustard (*phak-gard-dong*), salted mango (*ma-muang-dong*), and sweetened radish (*hua-chai-po*), obtained in Central and Eastern Thailand. These strains were placed in a monophyletic cluster consisting of *Lactobacillus*, *Pediococcus*, and *Leuconostoc* species and divided into seven groups based on phenotypic characteristics and 16S rRNA gene sequences. Eleven rod-shape strains of *Lactobacillus* were identified as *L. brevis* (Group I, 6 strains), *L. pentosus* (Group II, 2 strains), *L. fermentum* (Group III, 2 strains), and *L. plantarum* (Group IV, 1 strain). Two tetrad-forming strains of *Pediococcus* were identified as *P. acidilactici* (Group V, 1 strain) and *P. pentosaceus* (Group VI, 1 strain). In addition, three coccus chain bacteria were identified as *Leuconostoc mesenteroides* (Group VII, 3 strains). The produced GABA from the 16 strains ranged from 0.76 ± 0.02 to 13.42 ± 0.28 g/l. Among them, *L. brevis* GPB7-4 (Group I) gave the highest level of GABA production at 13.42 ± 0.28 g/l from 30 g/l monosodium glutamate (MSG) when cultivated at 30 °C for 72 h. Based on genome analysis, the GPB7-4 strain was closely related to *L. brevis* ATCC 367 with an ANIb value of 99.94%. The strain consisted of the complete genes of GABA producer *gadA* and *gadB* indicating its ability to produce GABA, thus it is a promising candidate for fermented food applications.

KEYWORDS: gamma-aminobutyric acid (GABA), lactic acid bacteria (LAB), *Lactobacillus brevis*, plant-based Thai fermented foods

INTRODUCTION

Gamma-aminobutyric acid (GABA) is formed during the decarboxylation of glutamic acid by glutamic decarboxylase (GAD, EC 4.1.1.15) [1]. GABA is a four-carbon non-protein amino acid that is widely distributed in plants, animals, and microorganisms. In animals, GABA functions as an inhibitory neurotransmitter in the brain, which directly affects personality and stress management, and as an antidepressant in various physiological functions [2], induction of hypotension [3], and cholesterol reduction [4]. A wide range of microbial fermented traditional foods contain GABA, which is considered safe and eco-friendly, and also offers novel health benefits [5, 6]. Several strains of lactic acid bacteria (LAB) can produce GAD and its biochemical properties have been characterized [7]. LAB are generally regarded as safe (GRAS) and their roles in the food and dairy industries, as bioactive-compound producing bacteria and as a probiotic, have been extensively studied [8]. However, most of researches on GABA production have focused on the production of microbials rather than the compound synthesis due to the corrosive nature of the reactant chemicals [9]. Many GABA-producing bacteria have been reported: *Lactobacillus delbrucekii* subsp. *bulgaricus* from cheeses; *L. brevis* from *Paocai* and *Kimchi*; *L. paracasei* from fermented fish (*fauna-sushi*) [5]; *L. rhamnosus* and *Streptococcus thermophilus* from raw milk cheese [6]; *L. buchneri* and *E. faecium* from *mukeunji kimchi* [10]; *Weissella hellenica* from *ika-kurozukuri* (salted squid); *Enterococcus ovium* from the bile of Holelithiasis patients; *E. faecalis* from retail ground beef; *L. fermentum* from raw milk; *Lactococcus lactis* subsp. *lactis* from the vaginas of healthy women [11]; *L. senmaizukei* from Japanese pickles [12]; *L. namurensis* and *Pediococcus pentosaceus* from *nham* (Thai fermented pork sausage) [13].

In Thailand, LAB refer to a large group of beneficial bacteria that are involved in lactic acid fermentation in foods [14]. Bamboo shoots (*naw-mai-dong*) were sliced and fermented in jars by adding

brine. *Phak-gard-dong* (pickled green mustard), packed tightly in jars, was fermented with a mixture of sugar and brine. This study focused on the screening, the identification, and the production of GABA by LAB isolated from plant-based fermented foods. Genome analyses of *gadA*/*gadB* genes of the selected GPB7-4 strain were determined and described.

MATERIALS AND METHODS

Isolation and screening of GABA-producing LAB using TLC

Sixteen samples were collected from plant-based fermented foods in Thailand: ten samples of *naw-mai-dong* from Chai Nat, Rayong, and Nakhon Nayok Provinces; four samples of *phak-gard-dong* from Samut Prakan and Lop Buri Provinces; and two samples, one each of *hua-chai-po* and *ma-muang-dong*, from the Bangkok Metropolitan. LAB were isolated by an enrichment culture approach (Table 2). Samples of 5 g were inoculated into 45 ml of de Man Rogosa and Sharpe (MRS) broth (Difco) in 50 ml Duran flasks and cultivated at 30 °C for 24 h. Pure cultures were obtained by streaking cultured cells on MRS-3% of $CaCO_3$ agar plate [15]. Colonies surrounded by a clear zone were picked and streaked on new MRS agar plates for purification. Pure cultures were maintained in MRS broth with 20% glycerol at −80 °C as a lyophilization technique for further study. Active cultures were prepared by a transfer of the pure culture in glycerol stock into 5 ml MRS broth and incubation at 30 °C for 24 h. Each inoculum with 10% (v/v) was transferred to a 10 ml of fermentation medium (GYP broth; 1% glucose, 1% yeast extract, 0.5% polypeptone, 0.2% sodium acetate, 20 ppm $MgSO_4 \cdot 7H_2O$, 1 ppm $MnSO_4 \cdot 4H_2O$, 1 ppm $FeSO_4 \cdot 7H_2O$, 1 ppm NaCl, pH 6.8) [16] supplemented with 1% monosodium glutamate (MSG) and cultivated at 30 °C for 72 h. The culture broth was centrifuged at 10 000 rpm for 5 min, and 2 µl of the supernatant was spotted onto thin-layer chromatography (TLC) plates (Aluminum sheet silica gel 60 F254 plates, Merck, Germany) for qualitative GABA analysis following the method described by Cho et al [10].

Identification of strains

Phenotypic characterization: Gram staining, cell morphology, and colonial appearance of the strains were examined using cells grown on MRS agar plates after incubation at 30 °C for 24 h. Physiological characteristics: growth at various pH values (3.5, 8.5, and 9.6), temperatures (15, 30, and 45 °C), and NaCl concentrations (6, 8 and 9% w/v); and biochemical activities: catalase activity, nitrate reduction, gas production, arginine hydrolysis, and acid formation from carbohydrates, were tested as described by Tanasupawat et al [17].

Genotypic characterization: DNA specimens were isolated and purified as reported by Saito and Miura [18]. For strains with difficulties in DNA isolation, a medium supplemented with 0.8–1.5% glycine was used [19]. The 16S rRNA gene sequences were amplified by polymerase chain reaction (PCR) using the primers 20F (5′-AGTTT GATCCTGGCTC-3′) and 1530R (5′-AAGGAGGTGAT CCAGCC-3′). Amplified 16S rRNA gene sequences were sequenced on a DNA sequencer (Macrogen, Korea) using universal primers including 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Multiple sequence alignments were performed using BLAST software from GenBank by BioEdit version 7.0.2. A phylogenetic tree was constructed by the neighbor-joining method using MEGA version 6 [20], and a bootstrap analysis was performed to determine confidence values of individual branches in the phylogenetic tree with 1000 replications [21]. Identified sequences were deposited in the DDBJ database.

Quantitative analysis of GABA using high-performance liquid chromatography (HPLC)

GABA production: Efficiency of GABA production from selected strains was assessed. Each inoculum with 10% (v/v) of 18 h old culture was transferred to the 10 ml of GYP broth supplemented with 3% of MSG and incubated at 30 °C for 72 h.

Analysis of GABA: To analyze GABA concentration, the standard and samples were centrifuged at 8000 rpm for 15 min, and the derivatized solution was injected into the HPLC system. The derivative method and mobile phase procedure followed Silva et al [22]. The HPLC instrument consisted of a Varian Prostar (Granite Quarry, NC, USA) Pump model 210. The fluorescence detector (FLD) model 363 was set at an excitation wavelength of 337 nm and an emission wavelength of 454 nm. The system was equipped with a 3 m particle size (150 mm × 4.6 mm, ID), C18 analytical column (Hibar-Futigsanle RT). GABA production test was conducted in triplicate and statistical analyses were performed using SPSS 18.0 software (IBM, USA). Analysis of variance was carried out using the ANOVA procedure, and the Duncan method was used to determine significant ($p \leqslant 0.05$) differences between mean values.

Genome annotation and alignment

Bacterial strains were cultivated in MRS broth as described above, and cells were harvested by refrigerated centrifugation at 10 000 × *g* (SIGMA 2K15 Centrifuge). Genomic DNA extraction was performed following a previously reported method [23]. Genome sequence of *L. brevis* GPB7-4 was implemented with the Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA) using 2 × 250 bp paired-end reads. The reads were

assembled to contigs using SPAdes 3.12 [24]. The draft genome of strain GPB7-4 is publicly available in GenBank (accession no. JAESIZ000000000). The genome was annotated using the RAST server [25], following the NCBI Prokaryotic Genome Annotation Pipeline (PGAP), and performed for comparison in the SEED Viewer [26]. Average nucleotide identities by BLAST (ANIb) values between strain GPB7-4 and related type strains were calculated using the JspeciesWS web-based tool [27]. Types of GAD genes were indicated as *gadA*;∼470aa, *gadB*:∼480aa, and *gadC*:∼500aa.

RESULTS AND DISCUSSION

Isolation and screening of GABA-producing LAB using TLC

Colonies of LAB exhibiting a clear zone on MRS agar plates containing 0.3% (w/v) $CaCO_3$, were isolated and purified. Sixteen strains showed the ability to produce GABA using qualitative analysis by TLC and compared with the GABA standard. The GPB7-4 strain, among the four strains on the TLC plates, produced the most prominent GABA spot (Fig. 1).

Identification of strains

Sixteen strains from 16 plant-based fermented foods were identified as Gram-positive, catalase-negative bacteria. Eleven rod-shaped strains included GPB5-2, GPB7-1, GPB7-4, GPB11-3, GPB16-4, GPB18-1, GPB20-2, GPB23-1, GPB23-3, GPD1-1, and FF2-2 with 5 cocci in pairs or tetrads as GPB23-5, GPK1-3B, PD-1, PD-3, and SP2-2. Their colonies on MRS agar plate were circular, low convex with an entire margin. All strains grew optimally at 30 °C and produced acid from L-arabinose, D-fructose, D-galactose, and D-glucose. Differential phenotypic characteristics of the isolated strains are shown in Table 1. The strains were placed in a monophyletic cluster, consisting of *Lactobacillus* and *Pediococcus* and *Leuconostoc* based on 16S rRNA gene sequence similarity, and divided into seven groups by phenotypic characteristics (Tables 1, 2 and Fig. 2).

Group I contained six strains of rod-shaped LAB (GPB7-1, GPB7-4, GPB11-3, GPB16-4, GPB18-1, and GPB23-3). These strains produced gas from D-glucose (heterofermentative LAB). They grew at pH 8.5, 45 °C, in 6 and 8% NaCl and produced acid from D-maltose and D-xylose. Their various characteristics are shown in Table 1. The results concurred with Phalakornkule and Tanasupawat [28]. The six strains showed high degree of 16S rRNA gene sequence similarity of 98.06–99.93% with *Lactobacillus brevis* ATCC 14869^T (Table 2 and Fig. 2).

Based on the 16S rRNA gene sequence of GPB7-4 (1566 bp), a representative of these strains was closely related to *L. brevis* ATCC 14869^T with 99.93% similarity (Table 2). Therefore, the strains from group I were identified as *L. brevis*. However, the name may be revised following the reclassification of *Lactobacillus* by Zheng et al [29], indicating that *L. brevis* should be replaced with the new scientific name of *Levilactobacillus brevis*.

Group II contained two strains of rod-shaped LAB (GPB20-2 and GPD1-1). These two strains did not produce gas from D-glucose (homofermentative LAB). They grew at pH 8.5 and 9.6 and in 6 and 8% NaCl. Besides, these two strains did not produce acid from D-raffinose (Table 1), as previously reported by Tanasupawat et al [30, 31]. Both the GPB20-2 (1358bp) and the GPD1-1 (1480bp) strains showed a high degree of 16S rRNA gene sequence similarity at 99.78–99.93% with *Lactobacillus pentosus* JCM 1558^T (Table 2). Therefore, they were identified as *L. pentosus*. Recently, the genus *Lactobacillus* was reclassified by Zheng et al [29], and *L. pentosus* was renamed *Lactiplantibacillus pentosus*.

Group III contained two strains of rod-shaped LAB, GPB5-2 and GPB23-1. These strains produced gas from D-glucose (heterofermentative LAB). They hydrolyzed arginine and grew at pH 3.5, at 45 °C; but not at pH 8.5 and 9.6 and not in 6, 8, and 9% NaCl. The strains did not produce acid from D-cellobiose, L-rhamnose, and D-sorbitol (Table 1), as reported by Phalakornkule and Tanasupawat [28], Tanasupawat et al [31], and Ahmad et al [32]. Both GPB5-2 (1270bp) and GPB23-1 (1476bp) strains showed a high degree of 16S rRNA gene sequence similarity at 99.92–99.93% with *Lactobacillus fermentum* CIP 102980^T (Table 2). Therefore, they were identified as *L. fermentum*. Recently, the genus *Lactobacillus* was reclassified by Zheng et al [29], and *L. fermentum* was renamed as *Limosilactobacillus fermentum*.

Group IV contained one strain of rod-shaped LAB, FF2-2. This strain did not produce gas from D-glucose (homofermentative LAB). Moreover, it neither hydrolyzed arginine nor grew at pH 9.6, in 9% NaCl. The strain produced acid from L-rhamnose and D-xylose (Table 1), as reported also by Phalakornkule and Tanasupawat [28], Tanasupawat et al [30] and Ahmad et al [32]. The FF2-2 (1395bp) strain showed a high degree of 16S rRNA gene sequence similarity at 99.93% with *Lactobacillus plantarum* NBRC 15891^T (Table 2). Therefore, it was identified as *L. plantarum*. Recently, the genus *Lactobacillus* was reclassified by Zheng et al [29] and *L. plantarum* was renamed as *Lactiplantibacillus plantarum*.

Group V contained one strain of tetrad cocci, GPB23-5. This strain: did not produce gas from D-glucose (homofermentative LAB); hydrolyzed arginine; grew at 45 °C, at pH 3.5 and in 9.6 and 6% NaCl; and produced acid from D-cellobiose, lactose, D-mannose, L-rhamnose, and D-xylose (Table 1) as reported also by Phalakornkule and Tanasupawat [28]. The GPB23-5 (1289bp) strain showed a high degree of 16S rRNA gene sequence similarity at 99.61% with

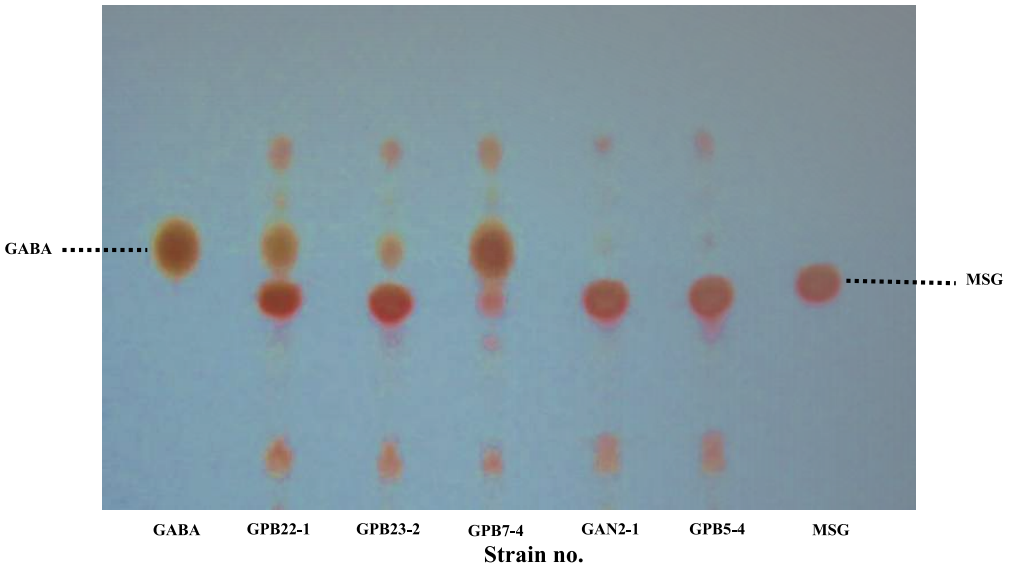

Fig. 1 TLC chromatogram of GABA production of representative strains. Lane 1, GABA standard (10 mg/ml); 2, strain GPB22-1; 3, strain GPB23-2; 4, strain GPB7-4; 5, strain GAN2-1; 6, strain GPB5-4; and 7, standard of monosodium glutamate (MSG) (10 mg/ml).

Table 1 Differential phenotypic characteristics of strains.

Characteristic	Group I (6)[a]	Group II (2)	Group III (2)	Group IV (1)	Group V (1)	Group VI (1)	Group VII (3)
Cell form	Rods	Rods	Rods	Rods	Cocci	Cocci	Cocci
Gas from glucose	+	−	+	−	−	−	+
Arginine hydrolysis	−	−	+	−	+	+	−
Growth at 45 °C	+	−	+	+	+	+	−
Growth at pH 3.5	−	−	+	+	+	w	−
Growth at pH 8.5	+	+	−	+	−	−	+
Growth at pH 9.6	−	+	−	−	−	−	−
Growth in 6% NaCl	+	+	−	+	+	+	+
Growth in 8% NaCl	+	+	−	+	−	+	+
Growth in 9% NaCl	w	−	−	−	−	−	+
Acid production from:							
D-Cellobiose	−	+	−	+	+	+	+
Lactose	−	+	+	+	+	−	+
D-Maltose	+	+	+	+	−	+	+
D-Mannose	−	+	+	+	+	+	+
D-Melibiose	w	+	w	+	−	−	+
D-Raffinose	−	−	+	+	−	w	+
L-Rhamnose	−	+	−	−	+	−	−
D-Sorbitol	−	+	−	+	−	−	−
Sucrose	−	+	+	w	−	+	+
D-Xylose	+	+	+	−	+	w	+

+, positive; w, weakly positive; −, negative reaction. [a] number of strains.

Pediococcus acidilactici DSM 20284^T (Table 2). Therefore, it was identified as *P. acidilactici*.

Group VI contained one strain of the tetrad cocci, GPK1-3B. This strain did not produce gas from D-glucose (homofermentative LAB). It hydrolyzed arginine, grew at 45 °C, at pH 6, and in 8% NaCl; but it did not produce acid from lactose, D-melibiose, L-rhamnose and D-sorbitol (Table 1) as reported also by Phalakornkule and Tanasupawat [28]. The GPK1-3B (1368bp) strain showed a high degree of 16S rRNA gene sequence similarity at 99.78% with *Pediococcus pentosaceus* DSM 20336^T (Table 2). Therefore, it was identified as *P. pentosaceus*.

Group VII contained three strains of coccal LAB;

Table 2 Samples' locations, strain number, group, 16S rRNA gene sequence similarity (%), accession number, and nearest type strain.

Sample	Province	Strain no.	Group	Similarity (%)	Accession no.	Nearest type strain
Naw-mai-dong	Chai Nat	GPB7-1	I	98.74	LC627488	*L. brevis* ATCC 14869^T
Naw-mai-dong	Chai Nat	GPB7-4	I	99.93	LC627489	*L. brevis* ATCC 14869^T
Naw-mai-dong	Rayong	GPB11-3	I	99.79	LC627490	*L. brevis* ATCC 14869^T
Naw-mai-dong	Chai Nat	GPB16-4	I	99.86	LC627491	*L. brevis* ATCC 14869^T
Naw-mai-dong	Chai Nat	GPB18-1	I	99.93	LC627492	*L. brevis* ATCC 14869^T
Naw-mai-dong	Chai Nat	GPB23-3	I	98.06	LC627493	*L. brevis* ATCC 14869^T
Naw-mai-dong	Chai Nat	GPB20-2	II	99.78	LC627494	*L. pentosus* JCM 1558^T
Phak-gard-dong	Samut Prakan	GPD1-1	II	99.93	LC627495	*L. pentosus* JCM 1558^T
Naw-mai-dong	Nakhon Nayok	GPB5-2	III	99.92	LC627496	*L. fermentum* CIP 102980^T
Naw-mai-dong	Chai Nat	GPB23-1	III	99.93	LC627497	*L. fermentum* CIP 102980^T
Ma-muang-dong	Bangkok	FF2-2	IV	99.93	LC627498	*L. plantarum* NBRC 15891^T
Naw-mai-dong	Chai Nat	GPB23-5	V	99.61	LC627499	*P. acidilactici* DSM 20284^T
Phak-gard-dong	Lop Buri	GPK1-3B	VI	99.78	LC627500	*P. pentosaceus* DSM 20336^T
Phak-gard-dong	Samut Prakan	PD-1	VII	99.86	LC627501	*Leu. mesenteroides* NBRC 100496^T
Phak-gard-dong	Samut Prakan	PD-3	VII	99.71	LC627502	*Leu. mesenteroides* NBRC 100496^T
Hua-chai-po	Bangkok	SP2-2	VII	99.93	LC627503	*Leu. mesenteroides* NBRC 100496^T

namely, PD-1, PD-3, and SP2-2. These strains produced gas from D-glucose (heterofermentative LAB). They did not hydrolyze arginine; they neither grew at 45 °C nor at pH 3.5 and 9.6; and they did not produce acid from L-rhamnose and D-sorbitol, as reported also by Tanasupawat et al [17]. The PD-1 (1461bp), PD-3 (1048bp) and SP2-2 (1337bp) strains showed a high degree of 16S rRNA gene sequence similarity at 99.71–99.93% with *Leuconostoc mesenteroides* NBRC 100496^T (Table 2). Therefore, they were identified as *Leu. mesenteroides*.

In Thailand, *Lactobacillus pentosus*, *L. plantarum*, *L. brevis*, *L. fermentum*, *L. sakei*, *L. farciminis*, *L. acidipiscis*, *L. pantheri*, *L. suebicus*, *L. thailandensis*, *L. camelliae*, *Pediococcus acidilactici*, *P. siamensis*, *P. pentosaceus*, *Tetragenococcus halophilus*, *Weissella confusa*, *W. cibaria*, *W. kimchi* and *Leuconostoc* sp. strains are distributed in fermented plant materials [33]. In this study, we found *L. brevis*, *L. pentosus*, *L. fermentum*, and *P. acidilactici* strains in *naw-mai-dong*; *L. pentosus*, *P. pentosaceus*, and *Leu. mesenteroides* strains in *phak-gard-dong*; *L. plantarum* in *ma-muang-dong*; and *Leu. mesenteroides* in *hua-chai-po*.

Quantitative analysis of GABA production using HPLC

GABA production of the 16 strains were screened on TLC plates and quantitatively determined using HPLC. The GABA standard curves showed a linear relationship ($R^2 = 0.9811$) between peak areas and GABA concentrations ranging from 2 to 64 mg/l of GABA. HPLC chromatograms of GABA standard solution and GABA produced after decarboxylation reactions by all the tested strains were obtained. GABA production of the 16 LAB isolates was determined in triplicate. The highest efficiency was shown by *L. brevis* GPB7-4 isolated from fermented bamboo shoots (*naw-mai-dong*), with maximum GABA concentration of 13.42 ± 0.28 g/l; while the GABA produced by the other 15 isolates ranged from 0.76 ± 0.02 to 13.27 ± 0.28 g/l (Fig. 3). Therefore, *L. brevis* GPB7-4 was selected for further study as a GABA production culture starter for the food industry. GABA production of *L. brevis* isolated from *kimchi* and pickled Chinese vegetables ranging 19.07–61 g/l was reported [34, 35]. Other species in the genus Lactobacillus also produced high GABA concentrations. *L. plantarum* NDC75017 isolated from a traditional fermented dairy product produced GABA at 3145.6 mg/kg substrate [36]; while *L. rhamnosus* GG, *Lactococcus lactis* subsp. *lactis*, *P. pentosaceus* NH102, and *P. pentosaceus* HN8 were recorded for GABA productions of 1.13, 3.68, 8.39, and 9.06 g/l, respectively [37].

Genome annotation and alignment

Genome features of strain GPB7-4 included 2 320 751 bp with a G+C content of 45.94%, 165X of genome coverage, 226 033 of contig N50, 3 of rRNA, 48 of contigs, and 2309 of the coding gene GPB7-4 exhibited ANIb value of 98.26% with *L. brevis* ATCC 367, and over the 95% threshold used to indicate strains as the same species [27]. Isolates of GABA-producing LAB in this study produced GABA at different levels. Strain GPB7-4 showed a high significant yield of GABA (Fig. 3). Therefore, genome sequencing is required to describe important genes for improvement of GABA production. To investigate the GAD genes, a maximum-likelihood tree was constructed with 1000 replications in the bootstrap test. The phylogenetic tree (Fig. 4) revealed that the GAD gene of *L. brevis* GPB7-4 (468aa) was closely related to *L. brevis* ATCC 367 (468aa), *L. brevis* WIKIM12 (468aa), *L. brevis* ZLB004 (468aa), *L. brevis* NCTC13386 (468aa), and *L. brevis* TMW

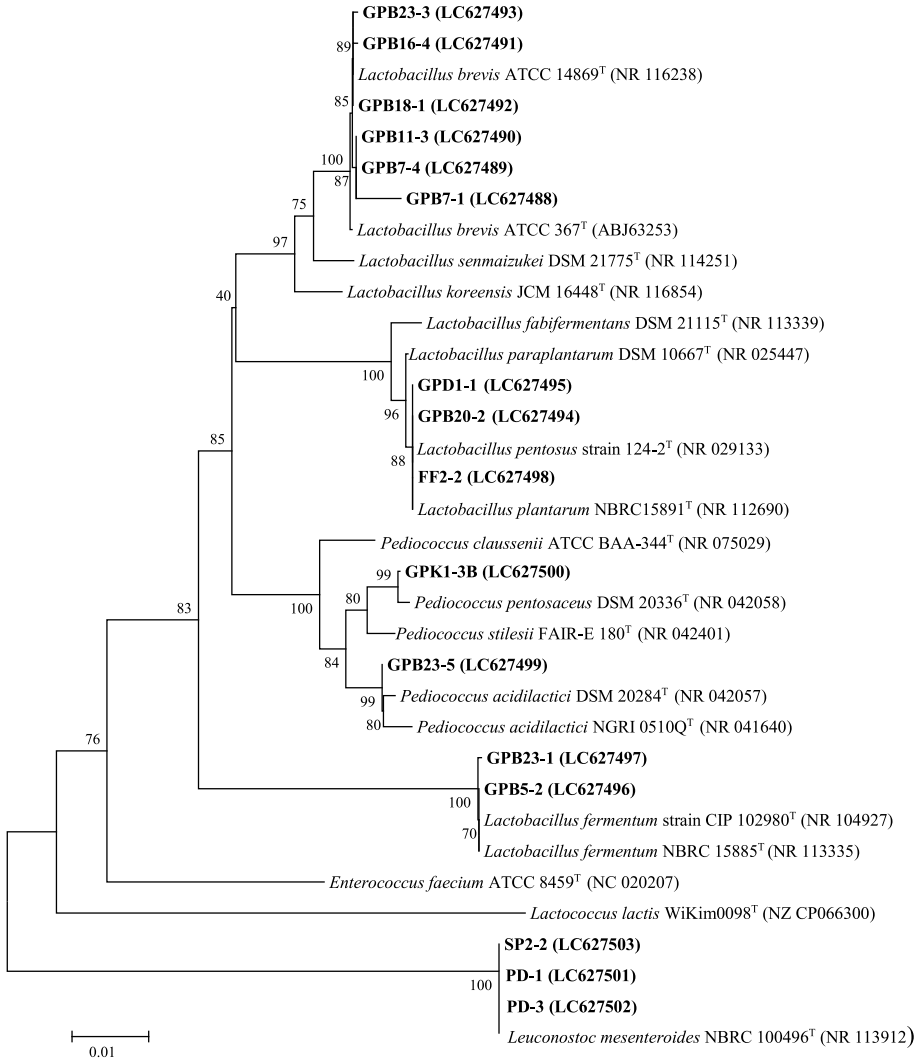

Fig. 2 Neighbor-joining tree based on 16S rRNA gene sequences showing relationships among *Lactobacillus*, *Enterococcus*, *Pediococcus*, and *Leuconostoc* strains and related species. Numbers on branches indicate percentage bootstrap values of 1000 replicates. Bar, 0.01 substitutions per nucleotide position.

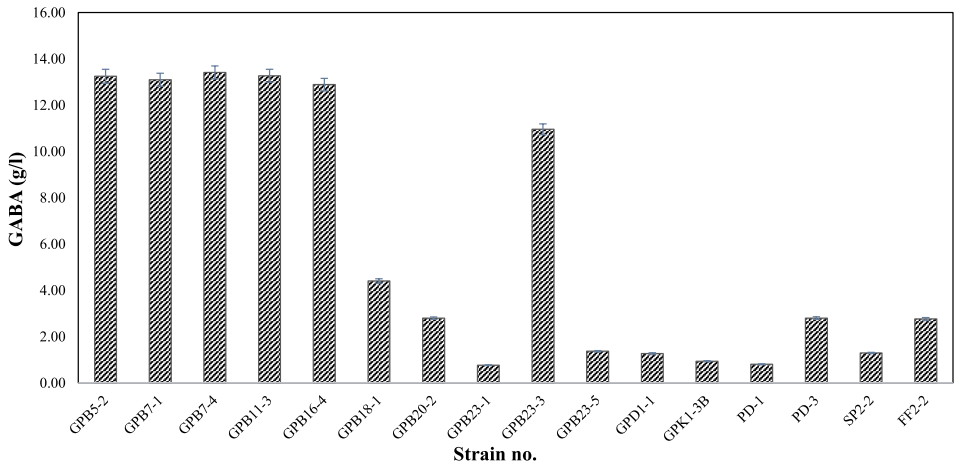

Fig. 3 GABA production (g/l) of strains cultivated in GYP with 3% monosodium glutamate (MSG) at 30 °C for 72 h.

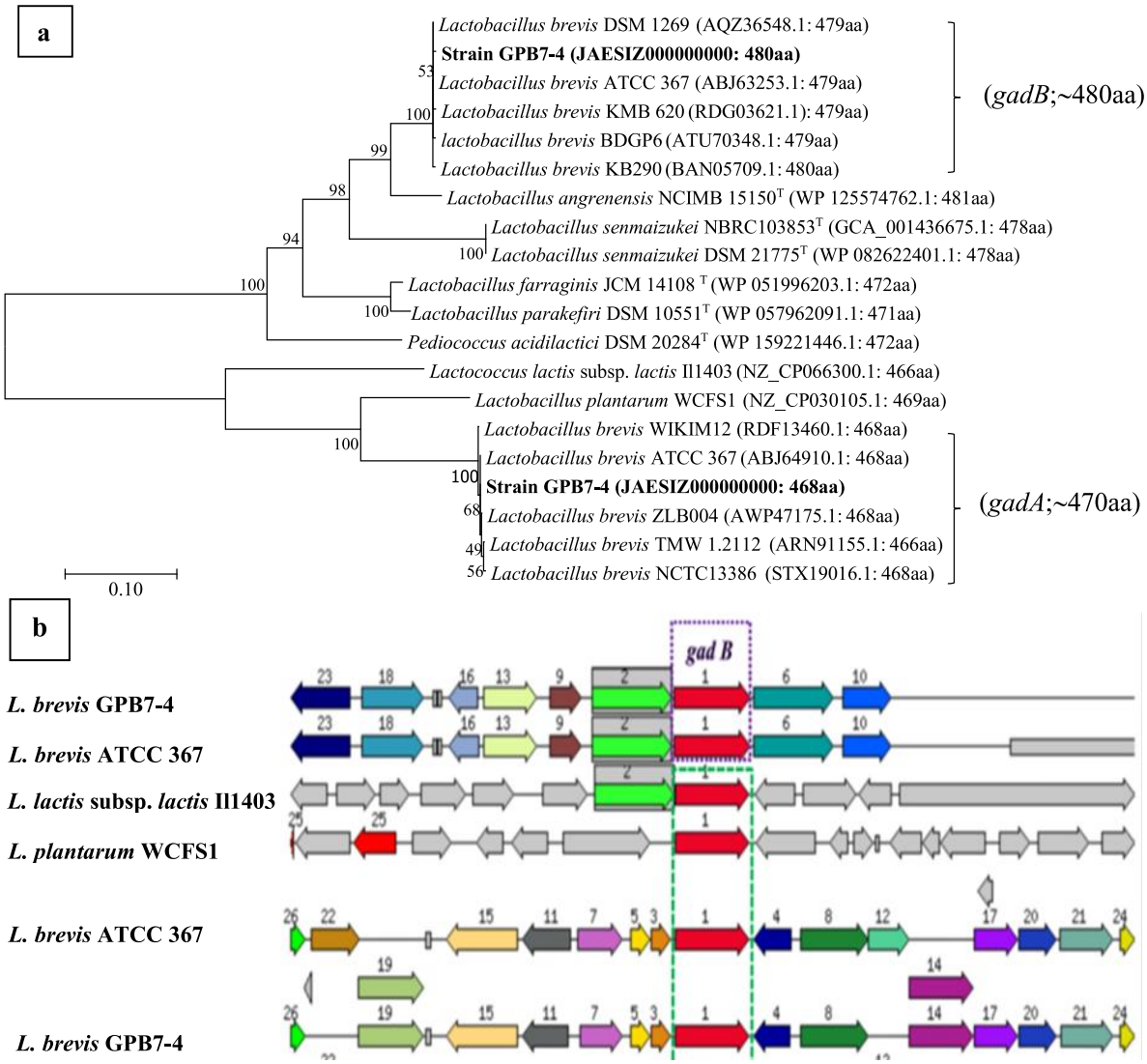

Fig. 4 Comparison of the GAD genes in *L. brevis* GPB7-4 and related strains. The maximum-likelihood tree is based on amino acid sequences of GAD genes. Numbers on branches indicate percentage bootstrap values of 1000 replicates. Length and GenBank accession numbers of the GAD genes from each strain are shown in brackets. Arrangements of GABA-producing genes (*gadA*/*gadB*) in *L. brevis* GPB7-4 and LAB strains are compared.

1.2112 (466aa) at 99.57 to 100% similarity; while the GAD gene of *L. brevis* GPB7-4 (480aa) was closely related to *L. brevis* ATCC 367 (480aa), *L. brevis* DSM 1269 (479aa), *L. brevis* KMB 620 (479aa), *L. brevis* BDGP6 (479aa), and *L. brevis* KB290 (480aa) at 99.58 to 99.79% similarity. Therefore, the comparative percentage of similarities of GAD genes encoding glutamate decarboxylase between *L. brevis* GPB7-4 and related GABA-producing strains indicated that *gadA* and *gadB* genes of *L. brevis* ATCC 367, *L. brevis* NPS-QW-145, and *L. brevis* CD0817 were the same and as previously reported [34, 38, 39].

CONCLUSION

Sixteen strains of GABA-producing LAB were isolated from plant-based fermented foods in Thailand. Strains from naw-mai-dong were identified as *L. brevis*, *L. pentosus*, *L. fermentum*, and *P. acidilactici*; while strains from *phak-gard-dong* were identified as *L. pentosus*, *P. pentosaceus*, and *Leu. mesenteroides*. The strains from *ma-muang-dong* and from *hua-chai-po* were identified as *L. plantarum* and *Leu. mesenteroides*, respectively. The effective strain *L. brevis* GPB7-4 produced high GABA (13.42 ± 0.28 g/l). The genome sequence of

L. brevis GPB7-4 exhibited *gadA*/*gadB* genes that were confirmed for GABA production. Further study is required to optimize GABA production and development for potentially functional food applications.

Acknowledgements: This research was supported by The National Research Council of Thailand (NRCT). We also thank the Institute of Research and Development, the Program in Food Science and Technology, Faculty of Agriculture and Life Sciences, Chandrakasem Rajabhat University and the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University for providing research facilities. Special thanks to Miss Nichanun Boonmak and Miss Pimthida Thammasang for technical assistance.

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