The γ-aminobutyric acid-producing ability under low pH conditions of lactic acid bacteria isolated from traditional fermented foods of Ishikawa Prefecture, Japan, with a strong ability to produce ACE-inhibitory peptides

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ABSTRACT

Many traditional fermented products are onsumed in Ishikawa Prefecture, Japan, such as *kaburazushi*, *narezushi*, *konkazuke*, and *ishiru*. Various kinds of lactic acid bacteria (LAB) are associated with their fermentation, however, characterization of LAB has not yet been elucidated in detail. In this study, we evaluated 53 isolates of LAB from various traditional fermented foods by taxonomic classification at the species level by analyzing the 16S ribosomal RNA gene (rDNA) sequences and carbohydrate assimilation abilities. We screened isolates that exhibited high angiotensin-converting enzyme (ACE) inhibitory activities in skim milk or soy protein media and produced high γ-aminobutyric acid (GABA) concentrations in culture supernatants when grown in de Man Rogosa Sharpe broth in the presence of 1% (w/v) glutamic acid. The results revealed that 10 isolates, i.e., *Lactobacillus buchneri* (2 isolates), *Lactobacillus brevis* (6 isolates), and *Weissella hellenica* (2 isolates) had a high GABA-producing ability of >500 mg/100 ml after 72 h of incubation at 35 °C. The ACE inhibitory activity of the whey cultured with milk protein by using *L. brevis* (3 isolates), *L. buchneri* (2 isolates), and *W. hellenica* (2 isolates) was stronger than that of all whey cultured with soy protein media, and these IC_{50} were < 1 mg protein/ml. Three of 10 isolates had high GABA-producing activities at pH 3, suggesting that they could be powerful candidates for use in the fermentation of food materials having low pH.

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1. Introduction

Ishikawa Prefecture in the Hokuriku region, which is located in the northern part of central Japan, is one of the major areas in which people produce various fermented fish products, such as fermented fish with cooked rice (*narezushi*), fermented fish and vegetable with malted rice (*kaburazushi*), fish fermented with rice bran mash (*konkazuke*), and fish sauce (*ishiru*). These products are known to be lactic acid fermented foods, in which various lactic acid bacteria (LAB) are involved in the production process [1–5].

Till date, many studies have demonstrated that several LAB species produce a ubiquitous four-carbon amino acid, γ-aminobutyric acid (GABA), which is synthesized from glutamic acid via a reaction catalyzed by glutamate decarboxylase [EC 4.1.1.15], a pyridoxal 5′-phosphate-dependent enzyme [6]. In mammals, GABA has various physiological functions, such as neurotransmission and the induction of hypotensive effects [7]. It is well known that it is involved in the regulation of cardiovascular functions such as blood pressure and heart rate, and that it plays an important role in the sensations of pain and anxiety [8]. In addition, GABA is found

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to have diuretic and tranquilizing effects [9]. Wong et al. [10] reported that GABA intake can help treat various neurological disorders such as Parkinson's disease, stiff-man syndrome, and schizophrenia. Although GABA is present in many fruits and vegetables, the amount of GABA present is very low, ranging from 0.03–2.00 μmol/g fresh weight [11]. Therefore, the development of new pharmaceutical and functional foods containing a considerable amount of GABA is required. Many studies have reported the mass production of GABA using *Lactobacillus brevis* isolated from alcohol distillery lees [12] and kimchi [13], *Lactobacillus paracasei* from fermented fish [14], and *Lactococcus lactis* from cheese starters [15].

Moreover, people have become increasingly interested in functional foods, because of a growing awareness among consumers of the link between diet and health. Angiotensin I-converting enzyme (ACE) [EC 3.4.15.1], a dipeptidyl carboxypeptidase, plays an important role in the regulation of blood pressure, and the enzyme cleaves the dipeptide portion from the C-terminal end of angiotensin I to produce the strong vasopressor angiotensin II. Hence, great interest has been shown in ACE-inhibitory peptides that have the ability to lower the blood pressure of hypertensive patients. Various ACE-inhibitory peptides have been isolated from different sources that are released during or after fermentation [16–19] or by hydrolytic processes [20].

The aim of this study was to screen various LAB exhibiting a strong ability to produce GABA and ACE inhibitors, which are expected to enhance the development of functional fermented foods. We tested both these abilities in LAB isolated from traditional fermented foods of Ishikawa Prefecture that were cultured in the presence of skim milk or soy protein. Based on this study, we discussed the possibility of these isolates being used as starters in the production of new types of fermented foods.

2. Materials and methods

2.1. Media and chemicals

De Man Rogosa Sharpe (MRS) medium was purchased from Oxoid (Basingstoke, Hampshire, UK). An API 50CH test kit was purchased from bioMérieux, Marcy l'Etoile, France. GABase (a mixture of γ-aminobutyrate glutamate aminotransferase and succinic semialdehyde dehyrogenase from *Pseudomonas fluorescens*) was purchased from Sigma-Aldrich (St Louis, MO, USA). NADP + was purchased from Oriental Yeast Co. (Tokyo, Japan). GABA, dithiotheriol, α-ketoglutarate, tris (hydroxymethyl) aminomethane (Tris), and sodium sulfate were obtained from Nakalai Tesque Inc. (Kyoto, Japan). Glutamic acid was purchased from Kanto Chemical Co. (Tokyo, Japan).

Table 1
LAB isolated from the fermented foods of Ishikawa Prefecture, Japan.

Fermented foods	Origin	Raw materials	Number of fermented food samples	LAB species identified (number of isolates)[a]
Narezushi	Purchased from market	Fish (horse mackerel, mackerel, or amberjack), rice, Japanese pepper, and red pepper	9	*Lactobacillus buchneri/ parabuchneri* (4), *Lactobacillus brevis* (5), *Lactobacillus alimentarius* (4), *Lactobacillus casei/paracasei* (2), *Lactobacillus plantarum/ paraplantarum* (7), *Pediococcus ethanolidurans* (1), *Weissella hellenica* (1)
Narezushi (our previous work [21])	Made by manufacturer	Fish (horse mackerel), rice, Japanese pepper, and red pepper	1	*Lactobacillus buchneri/ parabuchneri* (1), *Lactobacillus brevis* (2), *Lactobacillus plantarum/ paraplantarum* (1), *Lactobacillus casei/paracasei* (1),
Ika-koujizuke	Purchased from market	Squid and malted rice	1	*Carnobacterium divergens* (1), *Enterococcus faecalis* (1), *Enterococcus gilvus* (1), *W. hellenica* (1)
Ika-kurozukuri	Purchased from market	Squid and squid ink	1	*W. hellenica* (1)
Kaburazushi	Made by manufacturer	Yellow tail, turnip, and malted rice	1	*Leuconostoc mesenteroides* (1), *Leuconostoc citreum* (3), *Lactobacillus sakei* (3), *Lactobacillus curvatus* (1)
Daikonzushi	Made by manufacturer	Herring, Japanese radish, and malted rice	1	*Weissella cibaria* (1), *W. hellenica* (1), *L. citreum* (1), *L. mesenteroides* (2), *L. curvatus* (1), *L. sakei* (1), *Pediococcus pentosaceus* (1)
Yamahai-syubo	Made by manufacturer	Malted rice and cooked rice	1	*L. citreum* (1), *L. sakei* (2)

[a] Identified by 16S rDNA sequence analyses.

2.2. Isolation of LAB

LAB were isolated by spreading 10^1–10^4-fold diluted samples of various fermented foods of Ishikawa Prefecture onto MRS agar plates. Nine *narezushi* made from *aji* (horse mackerel, *Trachurus japonicus*), *saba* (mackerel, *Scomber japonicus*), or *buri* (amberjack, *Seriola quinqueradiata*) were purchased from different markets in Noto Peninsula, Ishikawa, Japan. Of 53 isolates shown in Table 1, five LAB were isolated in our previous work, and the *Lactobacillus brevis* strain ANP7-6 was isolated from *aji-narezushi* in that study (GenBank accession code AB666315) [21]. One *ika-koujizuke* (squid fermented with malted rice) and one *ika-kurozukuri* (squid fermented with squid ink) samples were also purchased from markets in Noto. *Kaburazushi* and *daikonzushi* were produced by Shijimaya-Hompo (Kanazawa, Ishikawa, Japan), while *yamahai-shubo* (a yeast mash used as a starter for Japanese sake) was produced by Syata-Shuzo (Hakusan, Ishikawa, Japan). *Kaburazushi*, *daikonzushi*, and *yamahai-shubo* were fermented in single tanks and the samples were withdrawn at various time points for isolation of LAB. From each fermented food sample, at least 5 colonies were randomly selected and used for species identification. Isolated colonies were streaked twice and stored in liquid culture at −80 °C in the presence of 15%–20% (v/v) glycerol. Details of the isolates from each food are summarized in Table 1.

2.3. Sequence analyses of the 16S ribosomal RNA genes

Genomic DNA was extracted from LAB cells using a Wizard Genomic DNA Extraction Kit (Promega, Madison, WI, USA). The entire 16S ribosomal RNA genes (rDNA) were amplified using the oligonucleotides F8 (5′-AGAGTTTGATCATGGCTCAG-3′) and R1510 (5′-ACGGYTACCTTTGTTACGACTT-3′) as a primer pair and the respective genomic DNA as templates. PCR was performed using ExTaq DNA polymerase (Takara, Shiga, Japan) with 25 cycles of denaturation (96 °C, 15 s), annealing (50 °C, 15 s), and extension (72 °C, 1 min and 30 s). The amplified fragments were purified using a QIAquick PCR purification Kit (Qiagen, Venlo, Netherlands) to remove the residual primers and were then used as templates in the next cycle for direct DNA sequencing. The sequence analysis was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3130 × 1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). At least 600 nucleotides that contained multiple hypervariable regions were analyzed to determine the taxonomic classification of LAB isolated at the species level. The 16S rDNA sequences of *L. brevis* (AN 1-5, AN 2-2, AN 3-5, AN 4-5, and SB 109), *Lactobacillus buchneri* (AN 1-1 and SB 21), and

Weissella hellenica (SB 101 and SB 105) were deposited in the DNA Data Bank of Japan under accession numbers LC136884-LC136892.

2.4. Preparation of the LAB cultures for biological tests

To identify LAB exhibiting a strong ability to produce GABA, respective isolates were cultivated in MRS medium containing 1% (w/v) glutamic acid at 35 °C for 1–5 days (time course). The medium was filtered through a membrane filter (0.45 μm), and the filtrate was stored at −20 °C until use. The amount of GABA in the filtrate was evaluated, the growth rate was determined, and the evolution of pH was also monitored. In addition, the amount of glutamic acid present in the medium after incubation and the amount of lactic acid were determined (see below). To evaluate the ability of the isolates to produce ACE inhibitors, LAB were cultivated in media that contained 1% (w/v) skim milk or soy protein in the presence of 10% (w/v) glucose. After fermentation, the whey was filtered (0.45 μm), and the filtrate was stored at −20 °C until use.

2.5. Measurement of the GABA contents

The GABA content of samples was determined by UV spectrophotometry using GABase based on changes in absorbance caused by the conversion of NADP+ to NADPH, as described previously by Tsukatani et al. [22]. In brief, the reaction mixture contained 80 mM Tris-HCl (pH 9.0), 750 mM sodium sulfate, 10 mM dithiothreitol, 1.4 mM NADP+, 2.0 mM α-ketoglutarate, and 30 μg/ml GABase and was prepared in a total volume of 90 μl. Subsequently, 10 μl of the standard solution (GABA) or samples was added to initiate the reaction at 30 °C for 2 h. The formation of NADPH was measured based on the change in the absorbance at 340 nm using a microplate reader. The absorbance of the blank prepared without GABase was spectrophotometrically measured, and the concentrations of GABA in the samples were calculated based on a calibration curve prepared using the standard solution. The amount of glutamic acid present in the medium was analyzed using a model L-8000 amino acid analyzer (Hitachi, Tokyo, Japan).

2.6. Determination of the ACE inhibitory activity

The ACE inhibitory activity was measured according to the method described by Nakano et al. [23], with some modifications. In brief, 0.025 U ACE (Sigma-Aldrich) was dissolved in 4.7 mM phosphate buffer (pH 8.5). A sample (25 μl) was incubated with the enzyme solution (50 μl) in the presence of the ACE substrate hippuryl-L-histidyl-L-leucine (Hip-His-Leu) at room temperature

Table 2
LAB with high GABA-producing abilities and ACE inhibitory activities.

Strain	LAB species identification based on the 16S rRNA sequence	Sequence homology with type strain	GABA, 96 h (mg/100 ml)[a]	ACE inhibition (IC$_{50}$) (mg protein/ml)[a]	
				Soya	Milk
AN 1–5	*L. brevis*	100%	616 ± 33	2.31 ± 0.06	0.57 ± 0.04
AN 2-2	*L. brevis*	100%	558 ± 11	9.44 ± 0.05	nd[b]
AN 3–5	*L. brevis*	100%	499 ± 15	1.46 ± 0.04	nd
AN 4–5	*L. brevis*	100%	721 ± 38	2.23 ± 0.04	nd
ANP 7-6	*L. brevis*	100%	657 ± 10	2.81 ± 0.11	0.83 ± 0.32
SB 109	*L. brevis*	100%	733 ± 43	2.36 ± 0.08	0.63 ± 0.04
AN 1-1	*L. buchneri*	100%	556 ± 44	1.33 ± 0.04	0.73 ± 0.02
SB 21	*L. buchneri*	100%	665 ± 27	1.59 ± 0.06	0.64 ± 0.04
SB 101	*W. hellenica*	100%	718 ± 33	1.37 ± 0.02	0.28 ± 0.01
SB 105	*W. hellenica*	100%	769 ± 21	1.42 ± 0.04	0.42 ± 0.02

[a] Data are the mean ± SD from three independent experiments.
[b] nd: Not detectable.

for 60 min. The reaction was then stopped by adding 0.3 M NaOH (750 μl) and 50 μl of 2% (w/v) o-phthalaldehyde dissolved in methanol. The mixture was kept at room temperature for 10 min, and subsequently, 100 μl of 3 M HCl was added. After incubation

for 60 min at room temperature, the amount of His-Leu liberated was determined by measuring the fluorescent intensity of its adduct with o-phthalaldehyde (excitation at 355 nm and emission at 460 nm). The magnitude of the inhibitory effect was expressed
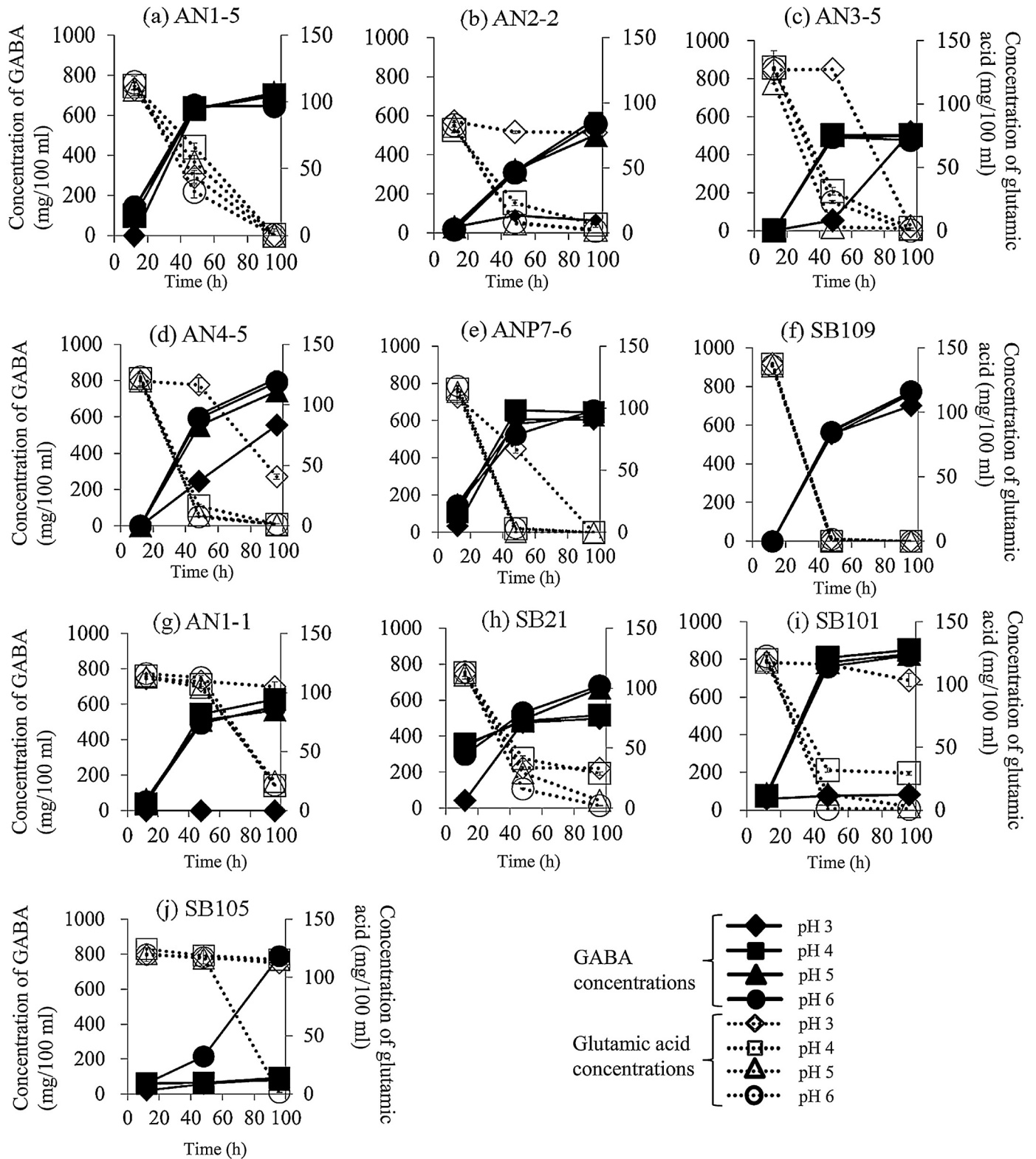

Fig. 1. The GABA-producing ability (filled symbols and solid lines) and utilization of glutamic acid (open symbols and dotted lines) at pH 3 (diamond), 4 (square), 5 (triangle), and 6 (circle) during cultivation of different strains isolated from traditional fermented foods in Ishikawa Prefecture, Japan. (a)–(f) *Lactobacillus brevis*, (g)–(h) *L. buchneri*, and (i)–(j) *Weissella hellenica*. The data points are the mean ± SD from at least three independent experiments.

as IC$_{50}$ (50% inhibitory concentration toward ACE, in mg protein/ml). Protein content was determined by the Bio-Rad protein assay kit (Bio-Rad laboratories, Hercules, USA).

2.7. Measurement of the lactic acid content

The amount of lactic acid produced during incubation was measured using a fast analyzer unit RQflex plus 10 and a Reflectoquant Lactic Acid Test (Merck, Darmstadt, Germany). The assays were based on the coupling reaction catalyzed by lactate dehydrogenase and diaphorase contained in the test strips for final formation of blue formazan, the concentration of which is determined reflectometrically.

3. Results and discussion

3.1. Screening of LAB producing GABA and their ACE inhibitory activity

We evaluated 53 randomly selected isolates from fermented foods using MRS medium to identify LAB that produced large amounts of GABA (Table 1). Of these, 5 isolates were previously obtained in our past work on *narezushi* [21]. The identification of strains involved 16S rDNA sequencing, and all strains shared 99%–100% identity with previously reported nucleotide sequences of each species. Among the isolates, *Lactobacillus* was dominant (35 isolates), and the other were *Leuconostoc* (eight isolates), *Weissella* (five), *Pediococcus* (two), *Enterococcus* (two), and *Carnobacterium* (one). These isolates were cultivated in MRS medium containing 1% (w/v) glutamic acid, and we measured the GABA concentration in the culture supernatant after filtration. As shown in Table 2, 10 LAB produced high amounts of GABA at a concentration of approximately 500 mg/100 ml or even higher (GABA/glutamic acid molar conversion ratio of >70%). Their 16S rDNA sequences shared 100% identity with typical strains in each genus, i.e., *Lactobacillus buchneri* NRRL B-30929 (GenBank accession number CP002652.1), *Weissella hellenica* NCFB2973 (NR_118771.1), and *L. brevis* ATCC14869 (AZCP01000010.1). We further investigated the assimilation potential of various sugars (by using API 50CHL) of these 10 isolates to precisely determine their taxonomic positions. Eight were the identical species based on classification using the 16S rDNA analyses, whereas SB101 and SB105 were identified as *Leuconostoc paramesenteroides*. The species *W. hellenica* has been defined as an individual subgroup of *L. paramesenteroides*, and the characteristics of these two species were very similar [24]. The 16S rDNA sequence analysis clearly indicated that SB101 and SB105 were assigned to *W. hellenica*; therefore, we regarded these isolates as the same species. There have been no reports so far that *W. hellenica* could produce GABA. A total of 8 of 10 isolates (SB21, SB109, AN1-1, AN1-5, AN2-2, AN3-5, AN4-5, and ANP7-6) were from *aji-narezushi* (salted and long-term fermented horse mackerel, *Trachurus japonicus*, with rice), which is a traditional fermented food popular in the Noto peninsula in Ishikawa Prefecture. The other two were from *ika-koujizuke* (SB101) and *ika-kurozukuri* (SB105). The ACE inhibitory activity (IC$_{50}$) of the whey cultured with milk protein media using 7 isolates (AN1-5, ANP7-6, SB109, AN1-1, SB21, SB101, and SB105) was low (< 1 mg protein/ml), suggesting that these LAB produce a strong ACE inhibitory peptide by decomposing milk protein during the fermentation. When these LAB were cultured with soy protein media, the whey from all isolates showed the ACE inhibitory activity. However, IC$_{50}$ of the whey was > 1 mg protein/ml and higher than that of the whey cultured with milk protein media. These results suggest that these LAB can utilize vegetable and animal sources for the production of ACE inhibitors. The summarized results are also shown in Table 2. All isolates

indicated in Table 2 are selected from different fermented food samples, so that we mentioned these as strains below.

3.2. GABA production under low pH condition

Yang et al. [25] reported that GABA biosynthesis by LABs is regulated by pH. Thus, we investigated the effects of different pH levels (pH 3, 4, 5, and 6) on the production of GABA during fermentation. The GABA-producing ability of the 10 strains was determined via a time course analysis of the extracellular GABA content (in the culture supernatant) (Fig. 1). The intracellular GABA content was remarkably low, which we determined by measuring the GABA content in the whole biomass of each strain (data not shown). The initial pH of the medium was adjusted to 3, 4, 5, and 6, and the pH was adjusted to their initial values every 12 h to evaluate the effects of different pH level on the GABA productivity.

Table 3
Lactic acid production by LAB strains at different pH values.

Strain	pH	Lactic acid (g/L)[a]		
		12 h	48 h	96 h
AN1-5 *L. brevis*	3	2.6 ± 0.2	7.9 ± 0.4	11 ± 1
	4	3.1 ± 0.2	19 ± 3	21 ± 3
	5	3.4 ± 0.2	17 ± 1	18 ± 2
	6	3.4 ± 0.6	19 ± 2	22 ± 2
AN2-2 *L. brevis*	3	1.4 ± 0.1	4.0 ± 0.3	7.3 ± 0.5
	4	2.1 ± 0.2	19 ± 1	15 ± 1
	5	2.0 ± 0.2	10 ± 1	15 ± 1
	6	2.0 ± 0.2	9.6 ± 0.9	12 ± 1
AN3-5 *L. brevis*	3	1.2 ± 0.1	8.4 ± 0.4	9.1 ± 0.3
	4	1.9 ± 0.3	11 ± 2	11 ± 2
	5	1.7 ± 0.3	15 ± 2	10 ± 3
	6	2.1 ± 0.2	9.5 ± 0.5	11 ± 2
AN4-5 *L. brevis*	3	1.3 ± 0.4	4.0 ± 0.6	10 ± 0
	4	1.8 ± 0.1	12 ± 4	11 ± 2
	5	1.9 ± 0.6	13 ± 3	11 ± 3
	6	1.9 ± 1.7	12 ± 3	18 ± 2
ANP7-6 *L. brevis*	3	2.7 ± 0.5	4.7 ± 1.9	9.8 ± 3.3
	4	3.1 ± 0.5	13 ± 2	17 ± 1
	5	3.0 ± 0.9	10 ± 1	22 ± 3
	6	2.5 ± 0.7	10 ± 3	14 ± 4
SB109 *L. brevis*	3	1.3 ± 0.1	4.9 ± 1.5	20 ± 3
	4	1.7 ± 0.3	12 ± 2	9.6 ± 1.5
	5	1.5 ± 0.2	11 ± 2	12 ± 3
	6	1.4 ± 0.2	9.5 ± 3.2	10 ± 3
AN1-1 *L. buchneri*	3	1.0 ± 0.2	2.3 ± 0.5	4.9 ± 0.7
	4	1.3 ± 0.3	5.4 ± 0.9	9.8 ± 1.2
	5	1.2 ± 0.1	6.3 ± 0.8	12 ± 2
	6	1.4 ± 0.5	5.4 ± 0.6	18 ± 2
SB21 *L. buchneri*	3	1.4 ± 0.4	6.8 ± 1.4	14 ± 2
	4	1.6 ± 0.2	12 ± 2	21 ± 3
	5	1.7 ± 0.4	15 ± 0	14 ± 2
	6	1.8 ± 0.3	13 ± 3	17 ± 1
SB101 *W. hellenica*	3	1.4 ± 0.2	1.6 ± 0.5	4.1 ± 1.1
	4	1.5 ± 0.2	12 ± 2	10 ± 4
	5	1.6 ± 0.4	22 ± 3	15 ± 4
	6	1.6 ± 0.3	19 ± 2	15 ± 4
SB105 *W. hellenica*	3	1.6 ± 0.1	1.5 ± 0.2	3.0 ± 0.5
	4	1.5 ± 0.2	5.8 ± 1.4	12 ± 2
	5	1.6 ± 0.2	12 ± 2	20 ± 2
	6	1.4 ± 0.3	16 ± 2	16 ± 2

[a] Data are the mean ± SD from three independent experiments.

As shown in Fig. 1a–j, the productivity reached to 200–800 mg/100 ml culture per approximately 50 h at neutral pH, which is almost comparable to that for *L. brevis* strain OPK-3 reported by Park and Oh [26]. pH had a marked effect on the GABA-producing ability. When the pH was adjusted to 3, the growth rate was low in some strains (data not shown), and the GABA production was significantly low compared with the values obtained when the pHs were adjusted to 4, 5, or 6 (Fig. 1b, AN2-2-*L. brevis*; 1 g, AN1-1-*L. buchneri*; 1i, SB101-*W. hellenica*; and 1j, SB105-*W. hellenica*). In particular, one strain SB105 (Fig. 1j) produced large amounts of GABA only when the pH was 6, whereas the amount of GABA produced was <100 mg/100 ml when the pH values were set to 3, 4 or 5. Three strains had a good ability to produce GABA, regardless of the pH value (Fig. 1a, AN1-5-*L. brevis*; 1e, ANP7-6-*L. brevis*; and 1f, SB109-*L. brevis*). All of these strains had a strong ability to consume glutamic acid within 96 h of the start of cultivation, and they produced maximum amounts of GABA even when the pH was 3. The other two strains (Fig. 1d, AN4-5-*L. brevis* and 1 h, SB21-*L. buchneri*) exhibited incomplete production of GABA at pH 3, although their ability to produce GABA at this pH was still obvious. In addition, we determined the lactic acid content of the medium during fermentation (Table 3). Three strains had lactic acid production levels that were <50% in the culture with pH 3 compared with pH 4–6, which indicated that their viabilities were reduced under low pH conditions (AN1-1, SB101, and SB105). This was because of the severe growth retardation effects caused by low pH, which almost correlated with the amount of GABA produced at pH 3 (Fig. 1). It is interesting to note that even in the strains that belonged to the same species, the ability to produce lactic acid varied over a wide pH range. One *L. brevis* strain SB109 produced lactic acid concentrations of >20 g/l at pH 3 within 96 h, whereas another AN2-2 strain had a weak ability to produce lactic acid and GABA (Fig. 1 and Table 3). These results suggest that we need to select the LAB starter at the strain level to prepare fermented foods derived from the production sources with a low pH.

In conclusion, we showed that LAB isolated from various fermented foods of the Ishikawa Prefecture of Japan could produce high amounts of biologically active compounds such as GABA and ACE inhibitors, which have many potential applications in functional foods. LAB had a key role in fermentation processes, and they have a long and safe history of application and consumption during the production of fermented foods and beverages. These strains could be used in the future as starters to produce new types of fermented foods or drinks with significant human health benefits, and they may facilitate the biosynthetic production of natural GABA. In particular, three *L. brevis* strains, AN1-5, ANP7-6, and SB109, had a strong GABA-producing ability at pH 3; therefore, they could be used effectively during fermented food production. New functional foods could be made from low pH production sources such as fruit juices, jam, or vinegar-containing beverages, which have not previously been used as candidate sources for LAB fermentation.

Acknowledgements

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