Gas release-based prescreening combined with reversed-phase HPLC quantitation for efficient selection of high- γ -aminobutyric acid (GABA)-producing lactic acid bacteria

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ABSTRACT

High γ -aminobutyric acid (GABA)-producing lactobacilli are promising for the manufacture of GABA-rich foods and to synthesize GRAS (generally recognized as safe)-grade GABA. However, common chromatography-based screening is time-consuming and inefficient. In the present study, Korean kimchi was used as a model of lactic acid-based fermented foods, and a gas release-based prescreening of potential GABA producers was developed. The ability to produce GABA by potential GABA producers in de Man, Rogosa, and Sharpe medium supplemented with or without monosodium glutamate was further determined by HPLC. Based on the results, 9 isolates were regarded as high GABA producers, and were further genetically identified as *Lactobacillus brevis* based on the sequences of 16S rRNA gene. Gas release-based prescreening combined with reversed-phase HPLC confirmation was an efficient and cost-effective method to identify high-GABA-producing LAB, which could be good candidates for probiotics. The GABA that is naturally produced by these high-GABA-producing LAB could be used as a food additive.

Key words: γ -aminobutyric acid, lactic acid bacteria, carbon dioxide, reversed-phase HPLC

INTRODUCTION

γ -Aminobutyric acid (**GABA**), a nonprotein amino acid, is the major inhibitory neurotransmitter of central nervous system; it is found mainly in the brain and regulates many physiological and psychological behaviors. Both GABA and GABA-rich foods have shown antianxiety and antidepressant activities (Krystal et al., 2002; Cryan and Kaupmann, 2005; Chuang et al., 2011), antihypertensive effects (Hayakawa et al., 2002; Inoue et al., 2003; Yoshimura et al., 2010; Liu et al.,

2011), and regulation of hormone secretion (Gamel-Didelon et al., 2002; Constantin et al., 2010). In China and Japan, generally recognized as safe (**GRAS**)-grade GABA has been approved as a functional food additive. Subsequently, GABA-rich foods have been developed by using GABA-producing microorganisms (Inoue et al., 2003; Rizzello et al., 2008; Sun et al., 2009; Yoshimura et al., 2010; Liu et al., 2011; Nejati et al., 2013) and are popular.

γ -Aminobutyric acid is widely distributed in mammals and plants but GABA is usually present in small amounts. Interest is growing in the use of GABA-producing microorganisms with food-grade status for manufacturing GABA-rich foods. High-GABA-producing lactic acid bacteria (**LAB**) are promising candidates as starter cultures for food fermentation and as cell factories for the synthesis of GRAS-grade GABA (Li and Cao, 2010). Recently, high-GABA-producing *Lactobacillus* species were applied in the dairy industry to manufacture GABA-rich yogurt and related products (Inoue et al., 2003; Sun et al., 2009; Liu et al., 2011; Nejati et al., 2013). So far, several strains of high-GABA-producing LAB have been isolated, including *Lactobacillus brevis* NCL912 from Chinese paocai (Li et al., 2008), *L. brevis* TCCC13007 from Chinese pickled vegetables (Zhang et al., 2012), *L. brevis* BH2, *L. brevis* K203, and *L. brevis* OPK-3 from Korean kimchi (Kim et al., 2007; Park and Oh, 2007; Binh et al., 2014), *L. brevis* DPC6108 from Irish infant feces (Barrett et al., 2012), *L. brevis* IFO12005 from Japanese alcoholic beverages (Yokoyama et al., 2002), *Lactobacillus paracasei* NFRI 7415 from Japanese fermented fish (Komatsuzaki et al., 2005), and *L. brevis* CGMCC 1306 from Italian unpasteurized milk (Fan et al., 2012). Species of *L. brevis* appear to show the most potential for producing large amounts of GABA (Li and Cao, 2010). Acid-based fermented foods, such as Korean kimchi and Chinese paocai, are natural habitats for GABA-producing LAB. This may be due to the presence of the glutamate-GABA system in these foods; this system reduces protons by decarboxylation of glutamate into GABA, which contributes to bacterial acid resistance (De Biase and Pennacchietti, 2012; Teixeira et al., 2014).

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Because GABA production from LAB is strain specific, screening of high-GABA-producing LAB from numerous isolates is time consuming. In previous reports, chromatography-based screening, such as HPLC, amino acid analyzer, and thin layer chromatography (TLC), were widely used (Li et al., 2008; Li and Cao, 2010). However, chromatography-based methods have some shortcomings, such as tedious sample preparation and low throughput, resulting in a time-consuming and inefficient process (Li and Cao, 2010). To date, only limited studies have been carried out to improve the screening for GABA-producing LAB. In the current study, Korean kimchi was used as a model of lactic acid-based fermented food, a prescreening method based on gas release was developed to identify potential high-GABA producers, and their GABA-producing ability was further determined by HPLC.

MATERIALS AND METHODS

Acid Treatment and Gas Release-Based Prescreening Procedure

The experimental process of acid treatment for harvested bacteria is shown in Figure 1. After acid treatment, the treated bacterial mixture was spread plated on to de Man, Rogosa, and Sharpe (MRS) agar and

incubated at 37°C for 24 h to obtain individual colonies. Selected colonies were inoculated in MRS medium containing 10 g/L of monosodium glutamate (MSG) in centrifuge tubes and statically incubated at 37°C for 24 h. Isolates that showed gas release after gentle pipetting and releasing the medium a few times were considered potential high GABA producers. Attention must be paid to this procedure to ensure that any air in the tip before pipetting is not introduced (air can be largely removed by pipetting down). The action of pipetting up and down must be slow and gentle; otherwise, bubbles will form because of the gas released from broth.

Fermentation Conditions for GABA Production

The ability to produce GABA by all potential high GABA producers was evaluated in MRS broth containing various concentrations of MSG. The MRS broth and MSG were autoclaved separately to avoid the Maillard reaction and reduction of MSG before mixing. One percent fresh bacterial culture (1×10^9 cfu/mL) was inoculated into 10 mL of MRS medium containing 0, 10, 30, 50, or 70 g/L of MSG and incubated aerobically at 37°C for 72 h. The concentration of GABA in MRS medium was monitored by reversed-phase HPLC every

Figure 1. Flow diagram of acid treatment of bacteria harvested from Korean kimchi and gas release-based screening procedure. MRS = de Man, Rogosa, and Sharpe medium; MSG = monosodium glutamate; GABA = γ -aminobutyric acid. Color version available online.

24 h. Three independent fermentations were carried out for each potential high GABA producer.

Determination of GABA Production by Reversed-Phase HPLC

Aliquots of MRS cultures were centrifuged at 12,000 $\times g$ for 10 min at room temperature to remove bacterial cells, and the supernatants were collected for dansyl derivatization. The preparation of dansyl AA was performed according to Le Vo et al. (2012). If the concentrations of MSG and GABA were too high, the supernatants were diluted 10-fold for the dansylation reaction.

Dansyl AA including dansyl GABA and dansyl glutamic acid were completely separated and quantified by reversed-phase HPLC (Shimadzu model LC-2010A, Shimadzu Corp., Kyoto, Japan) using a Kromasil 5- μ m 100A C18 column (250 mm $\times$ 4.6 mm; Phenomenex, Torrance, CA). Here, we developed a new separation program for dansyl AA by using 2 mobile phases: A (30 mM ammonium acetate, pH 7.5) and B (acetonitrile). The column was eluted with a linear gradient of 6 to 10% B over 0 to 5 min, 10 to 18% B over 5 to 7 min, 18 to 22% B over 7 to 15 min, 22 to 26% B over 15 to 18.5 min, 26 to 28.5% B over 18.5 to 22.5 min, 28.5 to 30% B over 22.5 to 24 min, 30 to 32% B over 24 to 27.5 min, 32 to 55% B over 27.5 to 40 min, 55 to 50% B over 40 to 45 min, 50 to 6% B over 45 to 48 min, and held at 6% B for 2 min. The flow rate of the mobile phase was 1 mL/min. Samples were injected at a volume of 20 μ L and were detected by absorbance of 275 nm. The oven temperature was maintained at 30°C.

16S rRNA Gene Sequencing

Genomic DNA from high GABA producers was extracted using ChargeSwitch gDNA mini bacteria kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Bacteria-specific primer 27F (5'-agagtttgatcatgcctcag-3') and universal primer 1492R (5'-ggttacctgttacgactt-3') were used to amplify the 16S rRNA gene (Marchesi et al., 1998). After amplification, PCR products were purified, ligated into T-vector, and transformed into competent *Escherichia coli* DH5 α . Successful transformants were chosen for Sanger sequencing after the selection procedure.

RESULTS

Gas Release-Based Prescreening

Based on the gas release phenomenon, 9 isolates were identified as potential high GABA producers from 500

randomly selected individual colonies. The principle for this screening method is production of CO₂ during decarboxylation of MSG (Figure 1). Because high GABA producers are rare in environments, it is not recommended to use chromatography-based techniques to determine the GABA yield in samples if all colonies are selected to inoculate in MRS broth containing MSG for incubation due to the substantial effort and high cost required for screening. This prescreening method based on gas release was efficient and cost effective in identifying potential high GABA producers.

Profiling of AA Using Reversed-Phase HPLC

Because there is a very small amount of glutamate in MRS medium, additional MSG is normally added as a substrate for GABA production. Separation and quantitation of 21 dansyl AA by reversed-phase HPLC was successful (chromatogram D, Figure 2). The production of GABA was increased by decarboxylation of MSG by high GABA producers in MRS medium supplemented with MSG (chromatograms A and B, Figure 2). A CO₂-producing *L. fermentum* W8 was used as a control for the gas release phenomenon. However, the production of CO₂ by *L. fermentum* W8 is mainly due to heterofermentation of glucose (Mayo et al., 2010). Its AA profile in MRS medium supplemented with 10 g/L of MSG after 24 h fermentation was evaluated but GABA production by *L. fermentum* W8 was not observed (chromatogram C, Figure 2). It was clear that *L. fermentum* W8 is not a GABA producer.

Effects of MSG Supplementation and Incubation Time on GABA Production

The production of GABA by 9 potential GABA producers at various MSG concentrations and incubation times is shown in Table 1. The bioconversion rates of all 9 LAB isolates were above 95.42% (with 10 g/L MSG and incubation time of 24 h), 78.77% (30 g/L MSG, 48 h), 62.45% (50 g/L MSG, 72 h), and 41.45% (70 g/L MSG, 72 h). In particular, isolate NPS-QW-255 showed the lowest conversion rate and isolate NPS-QW-171 showed the second lowest; however, their conversion rates were still high compared with the reported high-GABA-producing LAB strains (Li and Cao, 2010). A slight decrease in GABA was observed in most strains after 72 h of fermentation compared with that at 48 h.

Molecular Taxonomy of GABA Producers Based on the 16S rRNA Gene

The 9 potential high GABA producers were identified by 16S rRNA gene identification. Results of agarose gel

Figure 2. Representative reverse-phase HPLC chromatograms of AA profiles. A = de Man, Rogosa, and Sharpe (MRS) medium supplemented with 10 g/L of monosodium glutamate (MSG) before fermentation; B = MRS medium supplemented with 10 g/L of MSG after 24-h fermentation by isolate NPS-QW-281; C = MRS medium supplemented with 10 g/L of MSG after 24-h fermentation by *CO₂*-producing *Lactobacillus fermentum* W8; D = 0.1 g/L of each of 21 AA; peaks: 1 = aspartic acid; 2 = glutamic acid; 3 = asparagine; 4 = glutamine + arginine; 5 = serine; 6 = threonine; 7 = glycine; 8 = alanine; 9 = γ -aminobutyric acid (GABA); 10 = proline; 11 = valine; 12 = methionine; 13 = isoleucine; 14 = leucine; 15 = tryptophan; 16 = phenylalanine; 17 = cysteine; 18 = lysine; 19 = histidine; 20 = tyrosine. Color version available online.

electrophoresis are shown in Figure 3. The target length of partial 16S rRNA gene is ~1,465 bp. The BLAST analysis of 9 sequences of the 16S rRNA gene indicated that these 9 isolates were genetically identified as *L. brevis* (Table 2). The GenBank accession numbers for the 9 sequences are from KF851331 to KF851339.

DISCUSSION

Gas Release-Based Prescreening Is Associated with Bacterial Acid Resistance

Several important acid resistance systems, such as the F_0F_1 -ATPase system for discharging H^+ , amino acid deiminase system for neutralizing H^+ , and amino acid decarboxylase system for reducing H^+ , have been well demonstrated in bacteria (Lu et al., 2013; Teixeira et al., 2014). In addition, it has been well documented that the glutamate-GABA system contributes cell availability and metabolic activity for LAB during acidic

environments (De Biase and Pennacchietti, 2012). It is expected that acid-resistant bacteria may survive after exposure to acidic condition. With glutamate as the substrate in the medium, high GABA producers were able to survive in acidic conditions by reducing H^+ . Hence, high-GABA-producing LAB may be harvested after recovery by adjusting the pH to 4.0 for the test of gas release.

Time- and MSG Concentration-Dependent Bioconversion of Glutamate by High GABA Producers

Based on previous data on GABA production by *L. brevis* (Li and Cao, 2010), all 9 isolates in the present study were considered high-GABA-producing *L. brevis* strains (Table 1). Production of GABA is closely associated with membranal glutamate/GABA antiporter and cytoplasmic glutamate decarboxylase, as well as intracellular and extracellular acidity (Ma et al., 2012).

Table 1. Production of γ -aminobutyric acid (GABA; means $\pm$ SD) by potential high GABA producers in de Man, Rogosa, and Sharpe (MRS) medium supplemented with various concentrations of monosodium glutamate (MSG) at 37°C for 24, 48, and 72 h

Isolate	MSG (g/L)	GABA production (g/L)		
		24 h	48 h	72 h
NPS-QW-145	0	0.352 $\pm$ 0.021	0.401 $\pm$ 0.015	0.382 $\pm$ 0.012
	10	6.428 $\pm$ 0.752	6.628 $\pm$ 0.225	6.067 $\pm$ 0.543
	30	11.494 $\pm$ 1.244	18.258 $\pm$ 2.234	16.141 $\pm$ 0.947
	50	11.357 $\pm$ 0.628	22.693 $\pm$ 1.606	22.358 $\pm$ 2.302
	70	12.763 $\pm$ 0.690	23.402 $\pm$ 1.866	25.831 $\pm$ 1.898
NPS-QW-177	0	0.368 $\pm$ 0.004	0.373 $\pm$ 0.023	0.367 $\pm$ 0.041
	10	6.904 $\pm$ 0.926	6.496 $\pm$ 0.570	6.026 $\pm$ 0.463
	30	10.533 $\pm$ 0.758	17.508 $\pm$ 0.683	15.705 $\pm$ 0.877
	50	9.160 $\pm$ 0.629	21.949 $\pm$ 1.486	19.724 $\pm$ 1.691
	70	10.844 $\pm$ 0.874	24.098 $\pm$ 1.658	23.316 $\pm$ 2.760
NPS-QW-216	0	0.343 $\pm$ 0.045	0.444 $\pm$ 0.022	0.388 $\pm$ 0.052
	10	5.998 $\pm$ 0.257	6.515 $\pm$ 0.291	6.310 $\pm$ 0.770
	30	10.158 $\pm$ 0.860	16.812 $\pm$ 1.001	16.286 $\pm$ 1.365
	50	9.065 $\pm$ 0.250	20.171 $\pm$ 1.494	21.694 $\pm$ 1.365
	70	10.211 $\pm$ 0.518	21.286 $\pm$ 1.802	21.638 $\pm$ 3.159
NPS-QW-255	0	0.336 $\pm$ 0.010	0.361 $\pm$ 0.052	0.375 $\pm$ 0.035
	10	5.811 $\pm$ 0.189	5.696 $\pm$ 0.232	5.769 $\pm$ 0.340
	30	9.193 $\pm$ 0.727	14.407 $\pm$ 1.061	14.932 $\pm$ 1.227
	50	7.155 $\pm$ 0.320	15.761 $\pm$ 0.523	19.072 $\pm$ 0.668
	70	4.823 $\pm$ 0.173	15.405 $\pm$ 0.677	17.671 $\pm$ 1.557
NPS-QW-281	0	0.337 $\pm$ 0.008	0.326 $\pm$ 0.022	0.367 $\pm$ 0.001
	10	6.420 $\pm$ 0.188	6.227 $\pm$ 0.428	6.094 $\pm$ 0.463
	30	10.347 $\pm$ 0.108	16.293 $\pm$ 1.756	16.099 $\pm$ 1.927
	50	9.802 $\pm$ 0.557	19.719 $\pm$ 1.405	21.775 $\pm$ 1.857
	70	10.682 $\pm$ 0.683	22.089 $\pm$ 1.270	23.638 $\pm$ 2.041
NPS-QW-171	0	0.365 $\pm$ 0.029	0.300 $\pm$ 0.006	0.384 $\pm$ 0.027
	10	5.815 $\pm$ 0.183	5.438 $\pm$ 0.382	5.971 $\pm$ 0.165
	30	9.604 $\pm$ 1.223	14.387 $\pm$ 0.962	15.932 $\pm$ 0.760
	50	8.103 $\pm$ 0.835	17.614 $\pm$ 0.437	19.810 $\pm$ 0.954
	70	5.433 $\pm$ 0.141	16.087 $\pm$ 1.319	19.631 $\pm$ 0.904
NPS-QW-193	0	0.384 $\pm$ 0.006	0.381 $\pm$ 0.013	0.357 $\pm$ 0.029
	10	6.118 $\pm$ 0.709	6.729 $\pm$ 0.402	6.029 $\pm$ 0.459
	30	8.178 $\pm$ 0.800	16.490 $\pm$ 1.094	15.752 $\pm$ 0.911
	50	8.473 $\pm$ 0.876	19.614 $\pm$ 1.669	19.744 $\pm$ 1.653
	70	9.516 $\pm$ 1.395	22.112 $\pm$ 2.162	23.330 $\pm$ 2.800
NPS-QW-242	0	0.357 $\pm$ 0.014	0.326 $\pm$ 0.011	0.393 $\pm$ 0.031
	10	6.096 $\pm$ 0.006	6.281 $\pm$ 0.388	5.303 $\pm$ 0.394
	30	10.822 $\pm$ 0.906	17.773 $\pm$ 1.344	15.810 $\pm$ 1.368
	50	9.562 $\pm$ 0.220	19.535 $\pm$ 1.257	21.400 $\pm$ 1.822
	70	10.214 $\pm$ 1.167	22.986 $\pm$ 0.520	22.129 $\pm$ 2.075
NPS-QW-267	0	0.367 $\pm$ 0.010	0.320 $\pm$ 0.012	0.381 $\pm$ 0.016
	10	6.131 $\pm$ 0.395	6.434 $\pm$ 0.343	5.970 $\pm$ 0.496
	30	9.532 $\pm$ 0.529	16.857 $\pm$ 0.620	16.064 $\pm$ 0.338
	50	8.410 $\pm$ 0.124	19.464 $\pm$ 1.754	19.872 $\pm$ 2.365
	70	9.216 $\pm$ 0.160	21.825 $\pm$ 1.187	24.992 $\pm$ 2.334
Calculation of 100% conversion ¹	0		0	
	10		6.09	
	30		18.29	
	50		30.54	
	70		42.63	

¹100% conversion indicates that the MSG supplemented in MRS broth was completely converted into GABA.

As mentioned above, decarboxylation helps reduce intracellular H⁺ to maintain cellular viability during acidic conditions. As seen in Table 1, production of GABA increased during 72 h of fermentation with higher concentrations of MSG (50 and 70 g/L), whereas MSG was almost converted into GABA within 72 h at lower concentrations of MSG (e.g., 10 and 30 g/L). A

decrease in the concentration of GABA was observed for most strains at 72 h compared with that at 48 h, which may be related to degradation by GABA aminotransferase in *L. brevis* (Le Vo et al., 2012). Moreover, GABA yields from a GABA producer varied at the same time in MRS broth containing different concentrations of MSG (Table 1). This may suggest that increased Na⁺

Figure 3. Agarose gel electrophoresis of PCR products of 16S rRNA gene of 9 high γ -aminobutyric acid (GABA) producers.

from MSG affected cell viability and GABA production (Gandhi and Shah, 2014). Interestingly, we found that all potential high GABA producers were able to produce GABA in MRS medium without the addition of MSG. This occurred mainly because of the release of glutamate from proteins in the medium by bacterial proteolysis.

Accuracy of Gas Release-Based Prescreening of High GABA Producers

The gas from MRS medium after incubation could be CO_2 via heterofermentation of glucose or decarboxylation of glutamate or could be NH_3 production via deamination of AA (Mayo et al., 2010; Lu et al., 2013; Teixeira et al., 2014). However, the smell of released gas in the present study was not pungent, indicating that the main component of gas was CO_2 . It has been well documented that heterofermentative LAB are able to ferment glucose and lactose with lactic acid, acetic acid, ethanol, and CO_2 as by-products (Mayo et al., 2010). In present study, the LAB producing CO_2 could be high GABA producers or could be heterofermentative LAB. Based on the AA profiles of high GABA producers and CO_2 -producing *L. fermentum* W8 (chro-

matograms B and C, Figure 2), gas release was not a completely accurate method to identify high GABA producers. Although this prescreening seems to be empirical (based on visual observation), prescreening based on gas release is a way to identify potential high GABA producers.

Efficiency of Gas Release-Based Prescreening Combined with HPLC Confirmation

We compared our screening method with other methods, as shown in Table 3. In the present study, 9 isolates from 500 randomly selected colonies were identified as potential high GABA producers based on gas release and were further confirmed as high GABA producers by HPLC. Thus, efficiency from the prescreening step to HPLC confirmation was 100% because all potential high GABA producers were identified as high GABA producers after HPLC confirmation, which was very efficient compared with other methods (Table 3). In general, LAB isolates were individually cultured in MRS broth containing certain concentration of MSG before the prescreening step. Although prescreening based on observation of gas release may not be able to select LAB isolates that produce low levels of GABA, the method is efficient, rapid, simple, and cost effective compared with time-consuming and expensive chromatography-based prescreening techniques such as TLC and HPLC.

CONCLUSIONS

A high-throughput, rapid, simple, and cost-effective method for screening potential high GABA producers was developed based on gas release from Korean kimchi, and the GABA-producing capacity of these strains was confirmed by HPLC. In total, 9 LAB isolates were confirmed as high GABA producers by reversed-phase HPLC. These strains were genetically identified as *L. brevis* by 16S rRNA gene identification. This study provides a new method for screening high-GABA-producing LAB, which could be good candidates for probi-

Table 2. Alignment results of sequences of 16S rRNA gene of 9 high γ -aminobutyric acid (GABA) producers

Isolate	Closest species	Similarity (%)	GenBank Accession No. (reference sequence)
NPS-QW-145	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-171	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-177	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-193	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-216	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-242	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-255	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-267	<i>Lactobacillus brevis</i>	99	AP012167.1
NPS-QW-281	<i>Lactobacillus brevis</i>	99	AP012167.1

Table 3. Comparison of the efficiency of screening techniques for high γ -aminobutyric acid (GABA)-producing lactic acid bacteria (LAB)

Screening techniques ¹		Efficiency of screening high GABA producers			Reference
Prescreening	Confirmation	Origin of GABA producer	No. of randomly selected LAB isolates for screening steps	No. of GABA producers identified by prescreening or direct confirmation	
Gas release	HPLC	Korean kimchi	500	9	This study
NA	TLC	Korean kimchi	3,000	9	Park et al., 2014a
TLC	Spectrophotometer	Korean kimchi	Unclear	14	Binh et al., 2014
TLC	HPLC	Korean kimchi	Unclear	Unclear	Cho et al., 2007
TLC	HPLC	Korean kimchi	Unclear	Unclear	Cho et al., 2011
TLC	HPLC	Korean kimchi	Unclear	Unclear	Kim et al., 2007
TLC	AAA	Korean kimchi	Unclear	22	Lee et al., 2010
NA	AAA	Korean kimchi	Unclear	10	Lu et al., 2008
NA	Spectrophotometer	Korean kimchi	273	75	Park et al., 2014b
TLC	AAA	Myanmar fishery product	130	12	Thwe et al., 2011
NA	HPLC	Spanish cheeses	580	65	Diana et al., 2014
NA	AAA	Italian cheeses	440	61	Siragusa et al., 2007
TLC	HPLC	Chinese paocai	1,000	23	Li et al., 2008
NA	AAA	Human intestine	92	Unclear	Barrett et al., 2012
NA	AAA	Culture collection	72	Unclear	Komatsuzaki et al., 2005

¹NA = prescreening step not adopted; TLC = thin layer chromatography; AAA = amino acid analyzer.

²High GABA producers were defined here as microorganisms that converted at least 50% of supplemented monosodium glutamate into GABA during culture.

otics. These high-GABA-producing LAB isolates could be used as cell factories for manufacturing GRAS-grade GABA from food-grade bacteria.

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