1 **Production of γ -aminobutyric acid (GABA) by lactic acid bacteria strains isolated**
2 **from traditional, starter-free dairy products made from raw milk**
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29 13 **Running title: LAB producing GABA**
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34 15 **Key words:** Gamma-aminobutyric acid, GABA, functional foods, lactic acid bacteria,
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36 16 starters, adjunct cultures, dairy products
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Abstract

γ -Aminobutyric acid (GABA), an amino acid not used in protein synthesis, intervenes in several physiological functions and has both diuretic and calming effects in humans. Lactic acid bacteria (LAB) strains that produce GABA could be exploited for the manufacture of health-promoting GABA-enriched dairy products. In this study, 262 LAB strains isolated from traditional dairy products made from raw milk without starter cultures were screened for GABA production in culture media supplemented with 1% monosodium glutamate (MSG) using an enzymatic (GABase) method. About half of the strains (123) were found to be GABA producers. Of these, 24, among which were 16 *Lactococcus lactis* subsp. *lactis* and three *Streptococcus thermophilus* strains, produced >1 mM (range 1.01-2.81 mM) of GABA and were selected for further characterization. GABA production was confirmed in most strains by culturing in 5 mM MSG followed by HPLC quantification, although lower production levels were recorded by the latter method. Using species-specific primers, the gene encoding glutamate decarboxylase (GAD) was PCR-amplified in all but one of 21 of the GABA producers analysed. Amplicons sequences were compared to one another and to those held in databases. Except for one *Lactobacillus brevis* strain, none of the 24 GABA producers investigated produced toxic biogenic amines, such as tyramine, histamine or cadaverine. They were therefore considered safe. Either alone, in mixtures, or in combination with industrial starter and adjunct cultures, these strains might be useful in the development of health-orientated dairy products.

Introduction

γ -Aminobutyric acid (GABA), an amino acid not involved in protein synthesis, is produced via the decarboxylation of glutamic acid by the enzyme glutamate decarboxylase (GAD) (Dhakal et al., 2012). As a functional compound, it helps reduce blood pressure via the modulation of kidney function (Inoue et al., 2003), lessens psychological stress by acting like a tranquilizer (Croarkin et al., 2011), and might have anti-diabetic effects (Wan et al., 2015). Given these multiple health benefits, the food industry is interested in manufacturing GABA-enriched foods, including dairy products. Since casein is rich in glutamic acid, GABA can be abundant in milk. Its production in dairy products requires (i) the proteolysis of casein to release free glutamic acid as a precursor, and (ii) microorganisms (from starter cultures, adjuncts or from contaminating microbiota) with GAD activity (Linares et al., 2017; Dhakal et al., 2012). Alternatively, exogenous glutamate, usually in the form of monosodium glutamate (MSG), can be added to milk and then microbially converted into GABA (Linares et al., 2017).

GAD activity has been detected in different lactic acid bacteria (LAB), such as *Lactococcus lactis*, *Lactobacillus brevis*, *Lactobacillus paracasei*, and *Streptococcus thermophilus* (Nomura et al., 1998, Komatsuzaki et al., 2005; Siragusa et al., 2007; Lu et al., 2008). The decarboxylase activity of GAD seems to afford microorganisms greater resistance to acidic conditions - such as those found in fermented dairy products (cheese, yogurt, fermented milks, etc.) (Nomura et al., 2000). In some *Lactobacillus* species, decarboxylation seems to be coupled to energy biosynthesis (Higuchi et al., 1997); the conversion of glutamic acid into GABA therefore provides cells with both energy and pH resistance.

LAB strains with GAD activity could be used in cultures for making fermented, GABA-enriched, functional dairy products. However, no commercially available GABA-producing LAB starter or adjunct cultures is currently available (Parente and Cogan, 2004). Suitable strains safe to use in dairy food manufacture therefore need to be identified (Linares et al., 2017), and the search for them within the microbiota of traditional dairy products has been intensified (Renes et al., 2017; Franciosi et al., 2015; Diana et al., 2014; Siragusa et al., 2007). *Lactobacillus brevis* strains have been identified as among the most efficient of all GABA producers (Wu and Shah, 2016). However, they cannot be used as dairy starters since they are gas-forming heterofermenters and produce toxic biogenic amines (Linares et al., 2011). Promising results have been obtained, however, with a few *Lactococcus lactis* and *Streptococcus thermophilus* GABA producers, which produce this compound above its bioactivity threshold during cheese (Pouliot-Mathieu et al., 2013) and yoghurt (Linares et al., 2016) manufacture. However, the successful development of functional fermented dairy products requires the identification of more GABA-producing strains.

The GAD enzymes of several LAB species, and their encoding genes, have already been characterized. *Lc. lactis* GAD has an optimum pH of 4.7 and a molecular mass of 54 KDa (Nomura et al., 1998), and is encoded by a single gene forming part of an operon (*gadCB*) that codes for GAD and a GABA-glutamate antiporter (Nomura et al., 2000). The GAD enzyme of *Lb. brevis* has a molecular weight of 60 kDa, an optimum pH of 4.2, and is active as a dimer (Ueno et al., 1997; Park and Oh, 2007). GAD genes have also been cloned from *Lactobacillus plantarum* (Park and Oh, 2004), *Lactobacillus zymae* (Park et al., 2014) and *Lactococcus sakei* (Sa et al., 2015). GAD has yet to be detected in other technologically important species such as *Lactobacillus helveticus* or *Lactobacillus delbrueckii*, etc.

This work reports the screening of a large collection of LAB strains isolated from starter-free, traditional dairy products, in an effort to identify GABA-producing strains for their possible use in the development of functional dairy products. In addition, total DNA from the GABA producers was used as a template to amplify the GAD genes, and their sequences compared to those held in databases.

Material and Methods

Strains, media and culture conditions

GABA production was examined in 262 LAB strains belonging to the genera *Lactococcus* (153), *Lactobacillus* (54), *Streptococcus* (39), *Leuconostoc* (14), and others (2), isolated from traditional, starter-free dairy products made from raw-milk, i.e., cheeses (196 strains), yogurt (35), natural fermented milks (17), and kefir (14). Among these, 212 strains belonged to the most technologically-important species with respect to the formulation of dairy starters -153 *Lactococcus lactis* (120 *Lc. lactis* subsp. *lactis* and 33 *Lc. lactis* subsp. *cremoris*), 39 *Streptococcus thermophilus*, and 20 *Lactobacillus delbrueckii* (15 *Lb. delbrueckii* subsp. *bulgaricus* and 5 *Lb. delbrueckii* subsp. *lactis*). As a reference, a few LAB strains from the Belgian Coordinated Culture Collections of Microorganisms (BCCM/LMG) were also included. *Enterococcus faecalis* BA6y and *Lactobacillus parabuchneri* 2ES-1 (Ladero et al., 2015) were used as positive controls for the production of biogenic amines.

Lactobacilli were grown in MRS medium (Oxoid; Thermo-Fisher, Basingstoke, Hampshire, UK), while M17 medium (Oxoid) supplemented (1% w/v) with glucose (M17-Glu) or lactose (M17-Lac) was used for lactococci and *Streptococcus thermophilus*, respectively. *Leuconostoc* strains were grown in Elliker medium (Biokar;

Soalbia, Beauvais, France). For solid formulations of these media, 2% (w/v) of bacteriological agar (Oxoid) was added to the liquid media. Incubations were performed at 32 or 37°C for 24-48 h under aerobic or anaerobic conditions as required by the species under examination (Table 1).

Production of GABA by the GABase method

The GABA-producer screening method used in this work was based on that described by Tsukatani et al. (2005). Briefly, commercial GABase, a mixture of GABA-T (γ -aminobutyrate glutamate aminotransferase) and succinate semialdehyde dehydrogenase (Sigma-Aldrich), turns NADP⁺ (Sigma-Aldrich) into NADPH in the presence of GABA and α -ketoglutarate (Sigma-Aldrich). GABA was detected and quantified via the formation of this NADPH at 340 nm after incubation of the strains for 48 h under optimal conditions in appropriate liquid media (MRS, M17-Gly, M17-Lac or Elliker) supplemented with 1% monosodium glutamate (MSG) (Sigma-Aldrich, St. Louis, MO., USA). GABA production was estimated from calibration curves constructed with varying concentrations of GABA (Sigma-Aldrich) subjected to the same conditions as the cultures.

Production of GABA in cultures and HPLC quantification

Appropriate liquid media supplemented with 5 mM MSG were inoculated with the test strains and incubated for 48 h under optimal conditions. After centrifugation (13200 rpm for 10 min), the supernatants were filtered through a 0.45 μ m membrane (Millipore, Burlington, MA., USA). Aliquots of 100 μ L were then mixed with 175 μ L buffer (1M boric acid, pH 9.0), 75 μ L methanol, 3 μ L ethoxymethylene diethyl malonate (DEEMM; Sigma-Aldrich), and 2 μ L L-2-aminoadipic acid (Sigma-Aldrich) as an

internal standard. The mixtures were held at 30°C in an ultrasonic bath (Branson 221; Branson Ultrasonics, Danbury, CT., USA) for 45 min, and then incubated at 70°C for 2 h. Finally, they were filtered through a 0.2 µm cellulose filter (Millipore).

GABA was isolated by reverse-phase (RP)-HPLC in a Nova-pack C18 column (150 x 3.9 mm) using a Waters chromatograph controlled by Millenium 32 Software (Waters, Milford, MA., USA). Gradient elution of the GABA, and the detection and quantification conditions, were as previously reported (Fernández et al., 2007).

Amplification and sequencing of GAD genes

Total DNA from the test strains was extracted and purified using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich). The presence of GAD genes was determined by PCR amplification using species-specific primers based on available sequences: for *L. lactis* GadL-F 3'-ATGTTATACGGAAAAGAAAATC-5' and GadL-R 3'-TTAGTGAGTAAAGCCATATG-5', for *Lactobacillus plantarum* GadP-F 3'-ATGGCAATGTTATACGGTAAAC-5' and GadP-R 3'-GTGTGTGAATCCGTATTTCTTAG-5', and for *S. thermophilus* GadT-F 3'-ATGGATGAGAAGCTATTCAGAG-5' and GadT-R 3'-TTAATGATGGAAGCCGCTGCGATG-5'. Amplification conditions include an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 2 min, and a final extension step of 72°C for 10 min. Amplification was verified by agarose (2%) gel electrophoresis. Amplicons were visualized by ethidium bromide staining and then purified using Gen Elute PCR Clean-up columns (Sigma-Aldrich) and sequenced. The sequences obtained were assembled and compared to one another, and to those in databases using BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

DNA sequences were aligned using the MUSCLE program (Edgar, 2004) and their phylogenetic relationships calculated using the Approximate Likelihood-Ratio Test (Anisimova and Gascuel, 2006).

Production of biogenic amines

The production of tyramine, histamine, cadaverine and putrescine in liquid cultures in the presence of their corresponding precursor amino acids (tyrosine, histidine, lysine, and arginine or ornithine), was assayed after growing the strains in appropriate media supplemented with 2 mM of the above amino acids (all from Sigma-Aldrich) at the appropriate optimum temperature for 48 h. The cultures were then centrifuged at 8000 × g for 10 min, and the supernatants filtered through a 0.45 µm cellulose membrane. The resulting samples were derivatised and analysed by HPLC as above.

Results

Calibration curves for different GABA concentrations were constructed to estimate the amount of GABA produced by the LAB strains under study. To avoid interactions between components of the culture media and GABA, independent, medium-specific calibration curves were produced (for an example see the calibration curves for GABA in M17-Glu and MRS media in Supplementary Figure 1). Good linearity was seen between absorbance and the amount of GABA present in the media, although for any given concentration of GABA the absorbance was always higher in M17-Glu than in MRS. The absorbance of different GABA concentrations in M17-Lac and Elliker was similar to that recorded with M17-Glu (data not shown).

As shown by the GABase assay, the amount GABA produced by the different species and strains varied widely (from 0 to 2.61 mM). Of the 123 strains producing detectable amounts of GABA, 24 produced more than 1 mM (equivalent to 103.12 mg/L) (Figure 1; Table 1). Of these, all the *Lc. lactis*, *Lb. plantarum* and *Leuc. lactis* strains were originally isolated from cheese, while of the three *Streptococcus thermophilus* strains, two had been isolated from yoghurt and the other from a naturally fermented milk sample. All the *Lc. lactis* GABA-producers belonged to the *lactis* subspecies. To confirm the results, all 24 GABA producers (i.e., producers of >1 mM) were selected and tested for GABA production in media containing 5 mM of MSG. In this assay, GABA was detected and quantified by HPLC. After 48 h incubation at the corresponding optimum temperature, GABA production was confirmed for most strains but not all (Table 1). Surprisingly, the amounts of GABA recorded were always lower than those measured by the GABase assay. The strain recorded as producing the most by both methods was the collection strain *Lb. brevis* LMG 6906 (Table 1). *Lb. paracasei* L-PAR17 and *Lc. lactis* L-LAC13 and L-LAC39 produced GABA quantities similar to those of *Lb. brevis* LMG 6906 according to the GABase method, but no conversion of glutamate into GABA was detected by HPLC. Though the concentration of GABA recorded by the two methods employed was very different, several strains yielded detectable amounts in both assays, including 10 *Lc. lactis* strains, four *Lactobacillus* spp. strains, and one *S. thermophilus* strain (Table 1).

The presence of the GAD gene in *Lc. lactis*, *Lb. plantarum* and *S. thermophilus* strains was sought by PCR amplification, using species-specific oligonucleotide primers. Note that the *Lb. brevis*, *Lb. gasseri*, *Lb. paracasei* and *Leuc. lactis* strains were not tested; *L. brevis* has been abundantly studied and no genes have been made available for the other three species. Positive amplification was obtained for all the

strains tested, except for *Lc. lactis* L-LAC43 (Table 1; Figure 2). Internal segments encompassing a major part of all the amplified GAD genes were double-strand sequenced, and the sequences compared to one another, and to others in databases. The sequences (1260 bp) from all three *S. thermophilus* genes proved to be identical, and also identical or very similar to others held in the consulted databases (see below). In contrast, moderate heterogeneity was seen among the different genes of the *Lc. lactis* strains. This included nucleotide substitutions at 22 positions within a segment of 1238 bp out of the total 1398 bp of the GAD gene in this species. Figure 3 shows the phylogenetic relationships of the sequenced GAD gene segments from all the present *Lc. lactis* strains examined.

The availability of the genome sequences for two GABA-producing strains, *Lb. plantarum* LL441 (LWKN000000000) and *S. thermophilus* St-10 (PHHG000000000), allowed close inspections of the architecture of the GAD gene and its surrounding gene context (Supplementary Figure 2). The whole 1407 bp sequence of GAD from *Lb. plantarum* LL441 (locus tag A6B36_04600) showed just a single untranslated nucleotide change compared to that of *Lb. plantarum* HFC8 (Sequence ID CP012650.1), and proved to be highly similar to those of *Lb. plantarum* KACC 92189 (CP024058.1), SK151 (CP030105.1), AS-8 (CP028422.1), and many others. A gene encoding a putative YjeM-type glutamate/gamma-aminobutyrate family transporter protein (locus tag A6B36_06260) was identified in a different contig to that harbouring the GAD gene. Similarly, the GAD gene from *S. thermophilus* St-10 (locus tag CV716_08690) showed only two nucleotide differences to the sequences of *S. thermophilus* GABA (CP025399.1), APC191 (CP019935.1), and ND03 (CP002340.1). The GABA operon was located downstream of the gene encoding S-ribosylhomocysteine lyase (*LuxS*) and flanked by genes encoding the transposase-like

sequences IS1216 (upstream) and Tn1546 (downstream) (Supplementary Figure 2B).
Immediately downstream of GAD, a gene encoding the putative glutamate/gamma-aminobutyrate antiporter (GadC) of the GABA operon was also identified (locus tag CV716_08695).

With the exception of *Lb. brevis* LMG 6906, which produced tyramine from tyrosine (1.88 mM), none of the strains produced tyramine, histamine, cadaverine or putrescine from their precursor amino acids. In addition, the latter strain, plus four *Lc. lactis* strains (L-LAC1, L-LAC35, L-LAC73, and L-LAC75), produced small amounts of putrescine (0.03 to 0.80 mM) from arginine.

Discussion

The aim of this study was to screen for the ability to produce GABA in LAB strains of different genera and species isolated from traditional dairy products made from raw milk without the addition of starter cultures. Among these, a large number of the usual components of industrial dairy starters, such as *Lc. lactis*, *S. thermophilus*, and *Lb. delbrueckii*, were analysed. Wide variation in GABA production by the different LAB species and strains was seen. Variability in this phenotype has previously been reported for LAB from traditional dairy products (Renes et al., 2017; Franciosi et al., 2015; Diana et al., 2014; Siragusa et al., 2007). It is remarkable that all the *Lc. lactis* GABA-producers belonged to the *lactis* subspecies, since, under the test conditions, none of the 33 *Lc. lactis* subsp. *cremoris* was shown to produce any GABA. The GAD gene has been reported present in both *lactis* and *cremoris* subspecies. However, as a result of point mutations, in *Lc. lactis* subsp. *cremoris* it seems to be inactivated (Nomura et al., 2000). The smaller production of GABA in cultures supplemented with 2 mM might be

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268 due to the reduced amount of the precursor on the culture media, but also to losses
269 occurring throughout the laborious extraction and derivatisation processes required for
270 HPLC analysis. *Lb. brevis* LMG 6906 was always the best producer of GABA. This is
271 not surprising: strains of this species from different sources have been repeatedly
272 reported as GABA producers (Wu and Shah, 2016; Li et al., 2008; Siragusa et al.,
273 2007). Among the producers identified in this work, 10 *Lc. lactis* and three *S.*
274 *thermophilus* were found. Strains of these two species are the most economically
275 important components of commercial starters for industrial manufacture of dairy
276 products (Parente and Cogan, 2004). Other strains belonged to non-starter LAB species,
277 such as *Lb. paracasei*, *Lb. plantarum*, and *Leuc. Lactis*; these are frequently used as
278 commercial adjunct cultures (Settani and Moschetti, 2010). All GABA-producing
279 strains in this study were considered moderate producers. As compared to results in
280 other studies (Renes et al., 2017; Linares et al., 2016), among the tested strains GABA
281 overproducers were not detected. However, the present results agree well with the usual
282 amounts of GABA reported to be produced in culture media by LAB species from dairy
283 sources (Franciosi et al., 2014; Dhakal et al., 2012; Siragusa et al., 2007). As it has been
284 already reported for other strains (Lu et al., 2008), optimization of the medium and
285 culture conditions could be of help to improve GABA production during fermentation.

286 The production of GABA correlated well with the presence of the GAD gene, which
287 was detected by PCR amplification in all the strains tested except for *Lc. lactis* L-
288 LAC43. The absence of amplification in L-LAC43 might be due to sequence
289 heterogeneity within the positions of the primers. Several types of GAD-encoding genes
290 have been reported in LAB species from different origins (Yunes et al., 2016; Somkuti
291 et al., 2012; Nomura et al., 2000). Thus, the presence in L-LAC43 of a gene such as that
292 of *Lc. lactis* subsp. *lactis* ATCC 11454 (GenBank accession no. KF751353.2), which is

related to those found in *Lb. plantarum* and some *S. thermophilus*, cannot be ruled out. All GAD sequences from the three *S. thermophilus* strains were identical, while some modest sequence variation was seen for the gene from the *Lc. lactis* strains. Though small, this heterogeneity might still contribute towards differential GAD activity and, therefore, GABA production. The availability of genome sequences for the GABA producers *Lb. plantarum* LL441 and *S. thermophilus* St-10 allowed the genetic architecture of the two GAD loci to be examined. The results indicated that the genetic organization of the GAD gene in *Lb. plantarum* LL441 resembles that reported for other *L. plantarum* strains (Park and Oh, 2004), while the organization of the *S. thermophilus* St-10 GAD locus appears very similar to those of other studied *S. thermophilus* strains (Somkuti et al., 2012; Sun et al., 2012).

No relationship between the formation of GABA and biogenic amines was expected; independent enzymatic systems are involved in these processes (Dhakal et al., 2012; Linares et al., 2011). Nevertheless, checks were made to exclude harmful strains from entering into the food chain. No strains producing tyramine, histamine, cadaverine or putrescine (from ornithine) were detected. Some *Lc. lactis* produced putrescine from arginine by the agmatine deiminase (AGDI) pathway. In *Lc. lactis*, this route is thought to counteract acidification and is a species-specific trait (Del Río et al., 2016). However, during adaptation to the milk environment some dairy strains have lost this property (Ladero et al., 2011). Putrescine is not toxic, though it may enhance the toxicity of other biogenic amines (Del Río et al., 2017). Given these results, all the present GABA producers can be considered safe, except for the *Lb. brevis* strain.

Conclusions

This work examines the ability to produce GABA from MSG in LAB strains isolated from traditional dairy products made from raw milk without the use of commercial starters. Under the test conditions with 1% concentrations of the precursor amino acid, 24 strains were capable of producing more than 1 mM of GABA. Production in liquid cultures with 5 mM MSG was confirmed by HPLC for most strains. Using species-specific primers, the GAD gene was amplified by PCR in most of the GABA producers. The genetic organization of the GAD locus in two genome-sequenced strains was also analysed. The ability to produce GABA, associated with a series of suitable technological traits, provides a basis for exploiting selected LAB strains for the development of starter and adjunct cultures for making health-promoting dairy products. The production of GABA by selected strains and their mixtures under cheese and yoghurt manufacturing conditions is currently underway.

Acknowledgments

This work was partially supported by a project from the Plan for Science, Technology and Innovation of the Asturias Principality, co-funded by FEDER (Ref. GRUPIN14-137).

Declarations of interest

Authors have no conflicts of interest to declare.

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Figure Captions

Figure 1.- Production of GABA (mM) in liquid media supplemented with monosodium glutamate (1% w/v) by the bacterial species and strains used in this study.

Figure 2.- PCR amplicons of GAD genes from the *Lc. lactis* (A) and *S. thermophilus* (B) strains using species-specific primers. Order of strains, Panel A: M, molecular weight marker; 1, negative control; 2, L-LAC1; 3, L-LAC2; 4, L-LAC13; 5, L-LAC15; 6, L-LAC30; 7, L-LAC35; 8, L-LAC39; 9, L-LAC41; 10, L-LAC42; 11, L-LAC43; 12, L-LAC44; 13, L-LAC46; 14, L-LAC47; 15, L-LAC66; 16, L-LAC73; 17, L-LAC75. Panel B: M, molecular weight marker; 1, negative control; 2, S-THEF9.1; 3, S-THEF9.2; and 4, St-10; 3.

Figure 3.- Phylogenetic tree of an internal segment (1238 bp long) of the different GAD genes from the *Lactococcus lactis* subsp. *lactis* strains analysed in this study. The sequence of *Lc. lactis* subsp. *cremoris* MG 1363 (GenBank Accession no. AM406671.1; locus tag llmg_1179) was used as a comparator. The length of branches is proportional to the number of substitutions per site.

Figure 1

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Figure 2

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Figure 3

Table 1.- Selected strains producing >1 mM GABA in cultures supplemented with monosodium glutamate, culture conditions, and detection of GAD genes by PCR amplification.

Species	Strain	Medium and culture conditions	GABA production (mM) ^a		GAD gene amplification and sequencing
			Enzymatic method ^b	HPLC analysis ^c	
<i>Lactobacillus brevis</i>	LMG 6906	MRS/32°C/aerobiosis	2.81	2.60	nd
<i>Lactobacillus gasseri</i>	LMG 9203	MRS/37°C/anaerobiosis	1.35	0.10	nd
<i>Lactobacillus paracasei</i>	L-PAR17	MRS/37°C/aerobiosis	2.37	0.13	nd
<i>Lactobacillus plantarum</i>	LL441	MRS/32°C/aerobiosis	1.42	0.18	+
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	L-LAC1	M17-Glu/32°C/aerobiosis	1.43	0.59	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC2	M17-Glu/32°C/aerobiosis	1.93	0.53	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC13	M17-Glu/32°C/aerobiosis	2.14	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC15	M17-Glu/32°C/aerobiosis	1.80	0.95	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC30	M17-Glu/32°C/aerobiosis	1.31	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC35	M17-Glu/32°C/aerobiosis	1.47	0.65	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC39	M17-Glu/32°C/aerobiosis	2.39	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC41	M17-Glu/32°C/aerobiosis	1.01	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC42	M17-Glu/32°C/aerobiosis	1.16	0.30	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC43	M17-Glu/32°C/aerobiosis	1.24	0.49	-
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC44	M17-Glu/32°C/aerobiosis	1.17	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC46	M17-Glu/32°C/aerobiosis	1.05	0.23	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC47	M17-Glu/32°C/aerobiosis	1.62	0.21	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC66	M17-Glu/32°C/aerobiosis	1.21	-	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC73	M17-Glu/32°C/aerobiosis	1.14	0.51	+
<i>L. lactis</i> subsp. <i>lactis</i>	L-LAC75	M17-Glu/32°C/aerobiosis	1.30	0.45	+
<i>Leuconostoc lactis</i>	LEU-LAC1	Elliker/32°C/aerobiosis	1.13	-	nd
<i>Streptococcus thermophilus</i>	S-THEF9.1	M17-Lac/37°C/anaerobiosis	1.53	0.18	+
<i>S. thermophilus</i>	S-THEF9.2	M17-Lac/37°C/anaerobiosis	1.70	-	+
<i>S. thermophilus</i>	St-10	M17-Lac/37°C/anaerobiosis	1.43	-	+

^aResults are average of two biologically-independent replicates.^bGABA production in liquid cultures supplemented with 1% monosodium glutamate and quantified by an enzymatic method using GABase.^cGABA production in liquid cultures supplemented with 5 mM monosodium glutamate and quantified by HPLC analysis after derivatization.

nd, not done.

Supplementary Figure 1

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Supplementary Figure 2

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