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Aminotransferase and glutamate dehydrogenase activities in lactobacilli and streptococci

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ABSTRACT

Aminotransferases and glutamate dehydrogenase are two main types of enzymes involved in the initial steps of amino acid catabolism, which plays a key role in the cheese flavor development. In the present work, glutamate dehydrogenase and aminotransferase activities were screened in twenty one strains of lactic acid bacteria of dairy interest, either cheese-isolated or commercial starters, including fifteen mesophilic lactobacilli, four thermophilic lactobacilli, and two streptococci. The strains of *Streptococcus thermophilus* showed the highest glutamate dehydrogenase activity, which was significantly elevated compared with the lactobacilli. Aspartate aminotransferase prevailed in most strains tested, while the levels and specificity of other aminotransferases were highly strain- and species-dependent. The knowledge of enzymatic profiles of these starter and cheese-isolated cultures is helpful in proposing appropriate combinations of strains for improved or increased cheese flavor.

Introduction

Cheese flavor development is a dynamic and complex biochemical process, which results from catabolic reactions involving the main milk constituents, i.e., proteins, lipids, lactose, and citrate.[1] In particular, proteolysis and subsequent amino acid catabolism play significant roles in this process, regardless of the cheese variety. Essentially, it has been established that more than 50% of volatile compounds involved in the cheese flavor are produced via amino acid (AA) catabolism, and lactic acid bacteria (LAB) are mainly responsible for these reactions in most cheeses.[1,2] One of the main pathways that convert AAs into flavor compounds begins with a transamination reaction catalyzed by specific aminotransferases (ATs).[1,2] In this reaction, AAs are converted into their corresponding α-ketoacids due to the transfer of the α-amino group of an AA to a suitable acceptor, usually α-ketoglutarate, although pyruvate and oxaloacetate have also been reported as possible acceptors.[3,4] The α-ketoacids produced during transamination are intermediate compounds in the aroma development because they can be metabolized via a range of enzymatic and chemical reactions to provide several compounds that can have an impact on cheese flavor, such as alcohols, aldehydes, carboxylic acids, and esters.[1] In general, ATs are specific for an AA group, such as aromatic (Ar-AT) or branched-chain (Bc-AT) AAs, or for a single AA, such as aspartate (Asp-AT), although overlapping in their activities has been

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reported.[2,5–8] No specific AT for methionine has been isolated so far, but other ATs show activity for this amino acid.[6,7] Various ATs have been identified and characterized in *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*,[5,7,9] as well as in *Lactobacillus* spp.[8] In addition, it has been evidenced that transamination is the first step of the catabolism of several AAs in strains of *Lactobacillus casei*, *L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*.[10–12] Glutamate dehydrogenase (GDH) also plays a key role in transamination because it catalyzes the oxidative deamination of glutamate to α-ketoglutarate, providing an amino group acceptor.[2,6,12] Several species of LAB have demonstrated GDH activity, including *Lactobacillus* spp., namely, *L. casei*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, *L. pentosus*, and *L. acidophilus*,[13–16] *Lactococcus lactis*,[13,14,17,18] *Leuconostoc* spp.,[14,18] and *S. thermophilus*.[11]

The transamination step is considered the bottleneck in the AA catabolism and subsequent production of flavor compounds in cheeses. In particular, AT activities of starter and non-starter (NS) LAB, as well as the availability of an acceptor of the amino group, such as α-ketoglutarate, are important for the development of flavor in cheeses.[2,13] Similarly, Tanous et al.[13] have found that the ability of LAB to produce aroma compounds from AAs is closely related to their GDH activity, due to the production of an amino group acceptor in situ. On the other hand, it has been proposed that competition exists between ATs for the available α-ketoglutarate, and therefore AT activity profiles of lactic cultures may affect the volatile compounds produced in cheeses.[19] Thus, screening for enzymes playing key roles in cheese flavor development is relevant to the selection of strains as potential flavor-forming cultures.

This work aimed to study the profiles of ATs and GDH activities in twenty one LAB strains of dairy interest, including fifteen mesophilic lactobacilli strains (14 of NS origin and a wild strain), four strains of thermophilic lactobacilli (three strains isolated from whey cultures and one from a commercial source), and two commercial strains of *S. thermophilus*.

Materials and methods

Chemicals

L-Methionine (Met), L-tryptophan (Trp), L-tyrosine (Tyr), L-leucine (Leu), L-isoleucine (Ile), L-valine (Val), L-phenylalanine (Phe), L-aspartic acid (Asp), L-glutamic acid (Glu), α-ketoglutaric acid disodium salt dihydrate (α-KGA), pyridoxal 5′-phosphate (P5P), Tris–HCl, and Tris base were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Potassium phosphate monobasic and potassium phosphate dibasic were obtained from Anedra (Buenos Aires, Argentina). The Bradford protein estimation kit was from Pierce Chemical Company (Rockford, IL, USA), and the L-Glu assay kit was from R-Biopharm/Boehringer Mannheim (Germany).

Strains

Twenty-one strains of LAB were studied, including fifteen mesophilic lactobacilli, four thermophilic lactobacilli, and two streptococci. One of the mesophilic lactobacilli was a wild strain, *L. casei* BL23, whose genome had been sequenced.[20] The other 14 strains of mesophilic lactobacilli were isolated from two-month-old good quality Tybo cheese and belonged to the collection of our Institute (Instituto de Lactología Industrial, INLAIN). They were as follows: *L. plantarum* 29, 33, 87, 89, and 91, *L. rhamnosus* 73, 75, 77, and 78, *L. casei* 72, 81, and 90, and *L. fermentum* 28 and 46.[21] Three of the strains of thermophilic lactobacilli were isolated from whey cultures and also belonged to the INLAIN collection, including *L. delbrueckii* 133 and *L. helveticus* 138 and 209.[22,23] Finally, three commercial strains were studied, including *S. thermophilus* 1 and 2 and *L. helveticus* 3 (suppliers are not mentioned for confidentiality reasons).

Stock cultures of all strains were maintained frozen at −80 °C in appropriate broths supplemented with 15% (v/v) glycerol as a cryoprotective agent. Elliker broth (Biokar Diagnostics, France) was used for the streptococci, and MRS broth (Biokar Diagnostics) was used for the lactobacilli. Before use, the strains were grown overnight twice in their respective broths.

Growth curves

An overnight culture of each strain of the streptococci and lactobacilli was used to inoculate (2%, v/v) 100 mL of Elliker or MRS broth, respectively. The mesophilic lactobacilli were incubated at 37 °C, while the thermophilic lactobacilli and streptococci were incubated at 42 °C. Bacterial growth was monitored by assessing optical density (OD) at 560 nm in a spectrophotometer (UV/VIS Lambda 20, Perkin Elmer) at appropriate intervals to obtain growth curves and determine the late exponential growth phase for each strain.

Cell-free extract preparation

Cell-free extracts (CFEs) were obtained from cultures at the late exponential phase by mechanical disruption of the cells with glass beads (106 μm, Sigma, G8893) in a mini-beadbeater 8TM cell disruptor (Biospec Products, Bartlesville, IL, USA). In a preliminary experiment, we identified the best conditions for the beater operation to obtain a proper degree of cell disruption. For that, we assessed three different ratios of beads (g) to cells (mL) (1.2, 0.7, and 0.3 g/mL) and used three or five cycles of disruption, 1 min each, at the highest speed of the disruptor for a randomly selected strain, *L. plantarum* 91. The efficiency of cell disruption was quantified as the ratio between plate counts after and before the disruption.

Each strain was inoculated at 2% (v/v) and grown in the medium under the conditions described previously until it reached the late exponential phase, determined by the value of OD_{560} associated with this growth stage according to each growth curve. Cells were harvested by centrifugation (10,000 × *g*, 10 min, 4 °C), washed twice with 50 mM potassium phosphate buffer (pH 7.0), and suspended in an appropriate volume of the same buffer to give a 30-fold concentration of the cells. These suspensions were transferred to microtubes (2 mL), which contained different quantities of previously sterilized beads. CFEs were obtained under the following conditions established in the preliminary experiment: three cycles of 1 min each at the maximum speed of the beater, cooling on ice for 2 min between the cycles, and a bead/cell ratio

of 1.2 g/mL. A CFE consisted of the cell lysate centrifuged at 16,000 × *g*/10 °C for 15 min in order to separate the beads, cells, and cell debris and was then filtered through a 0.45-μm pore diameter membrane (Millipore, Sao Paulo, Brazil). The CFEs were stored at −20 °C until used for analysis of enzymatic activities. Duplicate CFEs were obtained from two independent cultures of each strain studied.

Determination of glutamate dehydrogenase activity

The GDH activity was determined colorimetrically by measuring the Glu-dependent reduction of NAD^+ in a coupled reaction with diaphorase using the commercial L-Glu assay kit.[24] A CFE (50–150 μL) was incubated in a reaction mixture containing potassium phosphate/triethanolamine buffer, pH 8.6, L-Glu, NAD^+, iodonitrotetrazolium chloride, and diaphorase (all provided in the L-Glu assay kit) for 1 h at 37 °C. Endogenous (Glu-independent) NAD^+ reduction by the CFE was assessed in control samples without Glu addition.[15] GDH activity was expressed per milligram of protein as the difference in absorbance at 492 nm between the sample and its respective control.

Determination of aminotransferase activities

AT activities against Asp, Met, branched-chain (Leu, Val, and Ile), and aromatic (Tyr, Trp, and Phe) AAs were analyzed by quantifying the Glu derived from a transamination reaction.

For this purpose, a CFE (15–50 μL) was incubated for 20 min at 37 °C in a reaction mixture (250 μL) containing 200 mM Tris buffer (pH 8.0), 1 mM P5P, freshly prepared 50 mM α-KGA, and 15 mM each AA substrate. In this reaction, an AA is transaminated to the corresponding α-ketoacid, and the α-KGA, which acts as an acceptor of the amino group, is converted to Glu. Endogenous Glu formation was assessed in control samples without the addition of a substrate AA. The reaction was stopped by heating at 80 °C for 15 min, and the level of the Glu produced was quantified with the same L-Glu kit described for GDH.[13,25,26] For that, an aliquot of the sample was incubated in a reaction mixture containing potassium phosphate/triethanolamine buffer, pH 8.6, NAD^+, iodonitrotetrazolium chloride, diaphorase, and GDH for 45 min at 37 °C.[26] Specific AT activity was expressed as micrograms of Glu produced per minute per milligram of protein.

Protein determination

The protein concentration of the CFE was determined by the Bradford method[27] with the Coomassie protein assay reagent provided by Pierce Chemical Company. Bovine serum albumin was used as an external standard. All protein determinations were performed in duplicate.

Statistical analysis

Analysis of variance (ANOVA) was applied to detect significant differences in the levels of AT activities against different AAs ($p < 0.05$). When differences were found, homogenous groups of means were identified (Duncan test). Principal component analysis (PCA), with standardization to a mean of zero and to a standard deviation of one (correlation matrix), was performed for the AT data in order to map cultures by their AA transformation abilities. Besides PCA mapping, hierarchical cluster analysis (HCA) was applied to the first two principal components to group the strains objectively in a score plot.

IBM SPSS Statistics 20.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis.

Results and discussion

Cell-free extract preparation

In the present work, we obtained CFEs by a mechanical disruption in a bead mill using the beads of 106 μm, which are optimal for bacterial cell disruption.[28] We performed preliminary experiments to optimize the beater operation conditions and found that the amount of disrupted cells increased significantly when the bead/cell ratio increased, which is attributed to an increased bead-to-bead interaction.[28] Contrarily, no difference in disruption was found depending on whether three or five cycles were applied. Based on these results, we used a bead/cell ratio of 1.2 g/mL for the subsequent experiments.

All cultures reached the end of the exponential growth phase between 8 and 12 h of incubation (data not shown), with values of OD_{560} from 1.6 to 2.1 (Table 1). The cell loads at harvesting were similar for all the strains tested, with the total average of 1.94×10^9 colony-forming units mL^{-1}. The cell disruption degrees were variable among the cultures and ranged between 74.4 and 99.8%, with a mean of 93% (Table 1). In addition, the protein content in the CFEs was 6.6 ± 1.7 mg mL^{-1} (data not shown).

Glutamate dehydrogenase and aminotransferase activities

Different levels of NAD-dependent GDH were detected in the strains assayed (Fig. 1). The two strains of *S. thermophilus* had the highest levels of GDH and were separated from the rest of the strains, especially *S. thermophilus* 2. The GDH levels in the other strains were lower and similar between the strains; the lowest activities were detected in the thermophilic lactobacilli and *L. casei* strains. Overall, the strains of *L. plantarum*, *L. rhamnosus*, and *L. fermentum* had intermediate levels of GDH. The GDH enzyme catalyzes the interconversion of α-ketoglutarate and Glu, with NAD or NADP as a cofactor. It has been reported that the cofactor for the anabolic enzyme during Glu production is the reduced form, NADP, while NAD is the cofactor for the catabolic enzyme involved in the Glu breakdown and subsequent production of α-ketoglutarate.[15] Helinck et al.[11] reported the presence of NADP-dependent GDH activity in five of six strains of *S. thermophilus*, while only one strain had NAD-dependent GDH activity. The presence and levels of GDH activity and its cofactor dependence have been found to be species- and strain-dependent in lactobacilli. In a group of isolates of NS origin, the highest frequency and activity of GDH were detected in *L. plantarum* strains.[15] Similarly, Tanous et al.[13] found higher levels of NADP-dependent GDH activity in *L. plantarum* strains in comparison with *L. casei* and *Lactococcus*. We found a similar trend, i.e., the highest levels of GDH were determined in the *L. plantarum*, *L. rhamnosus*, and *L. fermentum*

Table 1 – Cell loads for cultures in the broth before harvesting, in the buffer before and after the disruption treatment, and the efficiency of the disruption for each strain tested.

Species	Strain	Medium (MRS or Elliker)		Buffer		
		Optical density[a]	Cell counts (log CFU mL^{-1})[b]	Cell counts (log CFU mL^{-1})		Disruption (%)[d]
				Initial[c]	Final[c]	
L. plantarum	33	2.010	8.73	10.24	8.93	94.3
	91	2.020	9.25	9.30	7.18	99.2
	89	2.071	8.95	10.46	7.00	99.9
	29	1.971	8.51	10.50	9.02	94.3
	87	1.963	9.28	10.64	9.72	89.2
L. casei	81	1.960	9.17	10.56	9.93	74.4
	72	2.007	9.84	10.31	8.87	96.3
	BL23	2.080	9.65	9.45	7.70	99.0
L. paracasei	90	1.980	9.40	10.57	9.27	96.1
L. rhamnosus	73	1.847	9.46	10.15	8.04	99.2
	77	2.003	9.54	10.58	9.79	89.4
	78	1.958	9.22	10.74	8.08	99.8
	75	2.030	9.75	10.08	9.24	85.5
L. fermentum	28	1.985	8.99	9.51	8.00	96.7
	46	2.005	9.05	10.13	7.00	99.9
L. delbrueckii	133	2.056	8.93	10.33	8.24	99.1
L. helveticus	138	1.949	8.75	10.35	9.04	95.5
	209	2.059	8.79	10.15	8.08	99.2
	3	2.112	8.81	10.06	9.06	90.0
S. thermophilus	1	1.604	8.45	10.04	8.94	92.4
	2	1.793	9.57	10.65	9.88	75.6

The values represent the average of two independent experiments.

[a] Optical density of the cultures at harvesting.

[b] Cell counts in cultures at harvesting.

[c] Cell counts of the suspensions in potassium phosphate buffer before and after the disruption treatment with the bead mill.

[d] Percentages of cell disruption expressed as [(cell count before treatment – cell count after treatment)/cell count before treatment] × 100.

strains, while *L. casei* and *L. helveticus* showed lower activities. De Angelis et al.[16] also found the highest levels of GDH in *L. plantarum* strains, but the *L. casei* and *L. rhamnosus* strains that they characterized had similar levels.

On the other hand, the strains described in this study showed different levels of AT activity against Asp, Met, branched-chain, and aromatic AAs with α-ketoglutarate as an amino group acceptor (Table 2). AT specificity toward Asp was the main AT activity among the cultures studied, and its levels were 3–6 times higher than those of any other ATs specific for the AAs tested. The exceptions were *L. rhamnosus* 78, *L. casei* 72, and the four tested strains of thermophilic lactobacilli, in which activities of all ATs were similar or showed low absolute differences, without a prevalence of Asp-AT. Overall, strains that showed preferential AT activity against Asp had similarly low AT activities against the remaining AAs, with few exceptions. Thus, the second preferential AT activity after Asp-AT was against Tyr in *L. plantarum* 89 ($p < 0.05$). For *L. casei* 81, the second AA benefiting from AT activity was Val, while Trp, Phe, and Met were less transformed, and intermediate values were found for the remaining AAs. Finally, for *S. thermophilus* 2 the AT activity against Leu and Tyr was the second after Asp-AT. Similar to other authors,[2,8,29] we confirmed species and strain variability in AT profiles.

In the PCA of the levels of AT activities, two principal components explained 91.1% of the total variance. Fig. 2 shows the score and loading plots; the ellipses in the score plot enclose strains according to HCA. In the first group, three strains of *L. casei* (72, 81, and BL23), one strain of *S. thermophilus* (2), and one replica of *L. rhamnosus* 73 were grouped in the positive hemiplane of PC1. These strains were characterized by the highest activity of ATs. Among the strains clustered in this group, *L. casei* 72 had the lowest Asp-AT activity, which was evidenced by a negative score on PC2.

In the second group, located in the positive hemi-plane of PC2, there were three strains of *L. plantarum* (89, 33, and 91), and one replica of each one of the following four strains: *L. rhamnosus* 73 and 75, *L. paracasei* 90, and *L. plantarum* 87. These strains were characterized by the prevalence of Asp-AT activity.

Finally, the third group contained all strains of thermophilic lactobacilli (*L. delbrueckii* 133 and *L. helveticus* 138, 209, and 3), the two strains of *L. fermentum* (28 and 46), *L. rhamnosus* 77, and 78, *S. thermophilus* 1, *L. plantarum* 29, and one replica of each one of the following three strains: *L. plantarum* 87, *L. paracasei* 90, and *L. rhamnosus* 75. Overall, this group was placed on the negative sides of PC1 and PC2, which indicates that these strains had the lowest levels of ATs. Among the strains in this cluster,

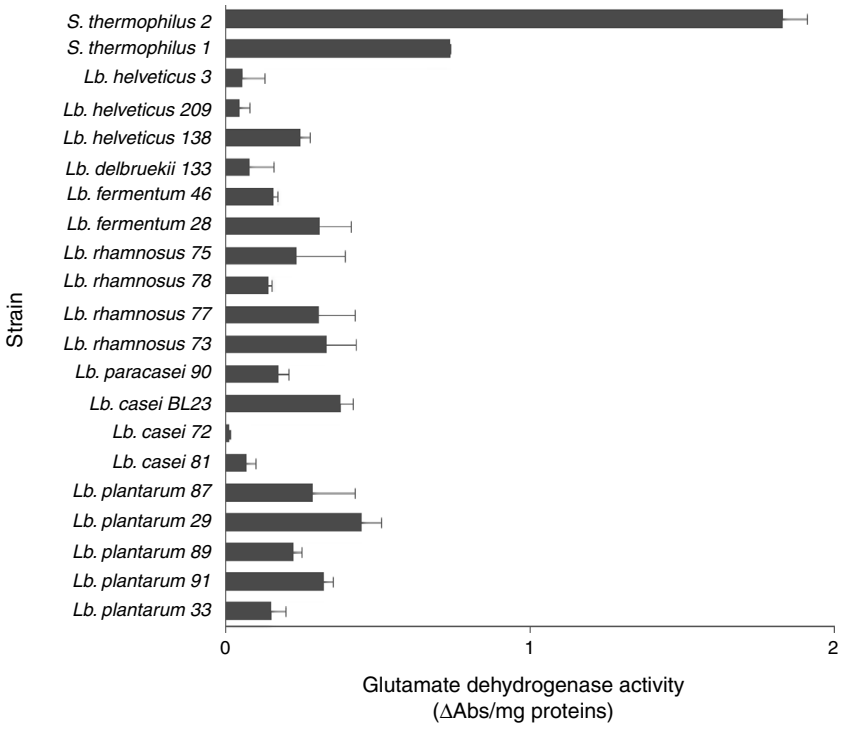

Fig. 1 – Glutamate dehydrogenase activity in the CFEs of the 21 tested strains. Values are means ± standard deviation of the results of two independent experiments.

Table 2 – Aminotransferase activities for eight amino acids.

Species	Strain	Asp	BcAA			ArAA			Met
			Leu	Val	Ile	Tyr	Trp	Phe	
L. plantarum	33*	7.46 ± 0.35^{a}	0.81 ± 0.11^{b}	0.84 ± 0.11^{b}	0.69 ± 0.06^{b}	0.74 ± 0.05^{b}	0.80 ± 0.05^{b}	0.85 ± 0.11^{b}	0.79 ± 0.17^{b}
	91*	7.36 ± 1.42^{a}	0.78 ± 0.04^{b}	1.08 ± 0.07^{b}	0.30 ± 0.07^{b}	1.36 ± 0.07^{b}	1.25 ± 0.07^{b}	1.25 ± 0.07^{b}	1.17 ± 0.07^{b}
	89*	9.46 ± 0.16^{a}	0.19 ± 0.01^{b,c}	0.08 ± 0.06^{c}	0.02 ± 0.01^{c}	0.69 ± 0.42^{b}	0.49 ± 0.21^{b,c}	0.54 ± 0.36^{b,c}	0.05 ± 0.01^{c}
	29*	3.12 ± 0.34^{a}	1.00 ± 0.62^{b}	1.02 ± 0.66^{b}	0.84 ± 0.65^{b}	1.12 ± 0.69^{b}	1.13 ± 0.64^{b}	1.06 ± 0.69^{b}	0.93 ± 0.70^{b}
	87*	6.53 ± 2.80^{a}	1.06 ± 0.36^{b}	0.83 ± 0.26^{b}	0.83 ± 0.25^{b}	1.16 ± 0.46^{b}	1.35 ± 0.65^{b}	1.28 ± 0.56^{b}	1.02 ± 0.51^{b}
L. casei	81*	7.18 ± 0.36^{a}	3.03 ± 0.18^{b,c}	3.31 ± 0.11^{b}	2.74 ± 0.06^{c,d}	2.31 ± 0.20^{d,e}	2.18 ± 0.07^{e}	1.92 ± 0.03^{e}	1.92 ± 0.35^{e}
	72	3.34 ± 0.52	2.61 ± 0.45	2.96 ± 0.67	2.73 ± 0.61	3.02 ± 0.52	2.90 ± 0.56	2.58 ± 0.56	2.07 ± 0.55
	BL23*	5.83 ± 0.71^{a}	1.95 ± 0.18^{b}	1.95 ± 0.23^{b}	1.34 ± 0.15^{b}	1.70 ± 0.36^{b}	1.53 ± 0.08^{b}	1.39 ± 0.05^{b}	1.65 ± 0.19^{b}
L. paracasei	90*	5.76 ± 2.34^{a}	1.34 ± 0.07^{b}	1.26 ± 0.26^{b}	1.89 ± 1.07^{b}	0.99 ± 0.49^{b}	1.86 ± 0.07^{b}	0.64 ± 0.37^{b}	0.86 ± 0.51^{b}
L. rhamnosus	73*	6.97 ± 1.75^{a}	2.50 ± 0.28^{b}	1.80 ± 0.12^{b}	1.65 ± 0.03^{b}	1.84 ± 0.09^{b}	2.43 ± 0.47^{b}	2.39 ± 0.41^{b}	2.25 ± 0.31^{b}
	77*	3.97 ± 0.21^{a}	1.19 ± 0.20^{b,c}	1.06 ± 0.17^{b,c}	0.89 ± 0.01^{c}	1.21 ± 0.11^{b,c}	1.19 ± 0.14^{b,c}	1.35 ± 0.25^{b}	0.88 ± 0.01^{c}
	78	1.51 ± 1.03	1.26 ± 0.52	0.96 ± 0.23	0.85 ± 0.10	1.17 ± 0.66	1.62 ± 0.75	1.70 ± 0.98	1.19 ± 0.04
	75*	4.33 ± 2.21^{a}	0.53 ± 0.32^{b}	0.57 ± 0.51^{b}	0.55 ± 0.40^{b}	0.53 ± 0.60^{b}	0.60 ± 0.43^{b}	0.62 ± 0.43^{b}	0.61 ± 0.26^{b}
L. fermentum	28*	3.42 ± 0.17^{a}	0.32 ± 0.10^{b}	0.30 ± 0.01^{b}	0.24 ± 0.03^{b}	0.38 ± 0.01^{b}	0.35 ± 0.04^{b}	0.34 ± 0.01^{b}	0.24 ± 0.02^{b}
	46*	3.36 ± 0.21^{a}	0.23 ± 0.14^{b}	0.21 ± 0.11^{b}	0.23 ± 0.01^{b}	0.21 ± 0.16^{b}	0.21 ± 0.13^{b}	0.23 ± 0.04^{b}	0.21 ± 0.01^{b}
L. delbrueckii	133*	0.81 ± 0.07^{b,c}	1.11 ± 0.03^{a}	0.99 ± 0.03^{a,b}	0.97 ± 0.07^{b}	0.89 ± 0.05^{b,c}	0.87 ± 0.04^{b,c}	0.92 ± 0.07^{b,c}	0.86 ± 0.07^{b,c}
L. helveticus	138*	1.20 ± 0.24^{a,b}	1.29 ± 0.15^{a,b}	1.12 ± 0.09^{b}	1.29 ± 0.29^{a,b}	1.77 ± 0.39^{a}	1.23 ± 0.24^{a,b}	1.25 ± 0.28^{a,b}	1.16 ± 0.22^{a,b}
	209	1.21 ± 0.15	1.71 ± 0.21	1.44 ± 0.32	1.52 ± 0.18	1.45 ± 0.32	1.14 ± 0.09	1.14 ± 0.29	1.18 ± 0.10
	3	1.02 ± 0.45	0.99 ± 0.38	1.06 ± 0.41	1.17 ± 0.54	1.37 ± 0.51	1.04 ± 0.48	0.98 ± 0.44	1.14 ± 0.51
S. thermophilus	1*	3.04 ± 0.01^{a}	0.90 ± 0.07^{b}	0.79 ± 0.10^{b}	0.74 ± 0.14^{b}	0.91 ± 0.06^{b}	0.81 ± 0.02^{b}	0.89 ± 0.06^{b}	0.72 ± 0.10^{b}
	2*	5.78 ± 0.73^{a}	4.37 ± 0.41^{b,c}	2.90 ± 0.33^{d}	2.53 ± 0.07^{d}	4.70 ± 0.66^{b}	3.00 ± 0.07^{d}	3.40 ± 0.50^{c,d}	3.03 ± 0.48^{d}

Asp, branched-chain amino acids (BcAA: Leu, Val, Ile), aromatic amino acids (ArAA: Tyr, Trp Phe), and Met, in CFE of the 21 strains tested. Activities are expressed as μg of glutamic produced from α-ketoglutarate per minute and milligram of protein.
Values are the means ± standard deviation of the results of two independent experiments.

* Strains that showed significant differences in ATs activity toward different AA ($p < 0.05$).
Values with different superscripts (a, b, c, d and e) within a row are significantly different ($p < 0.05$).

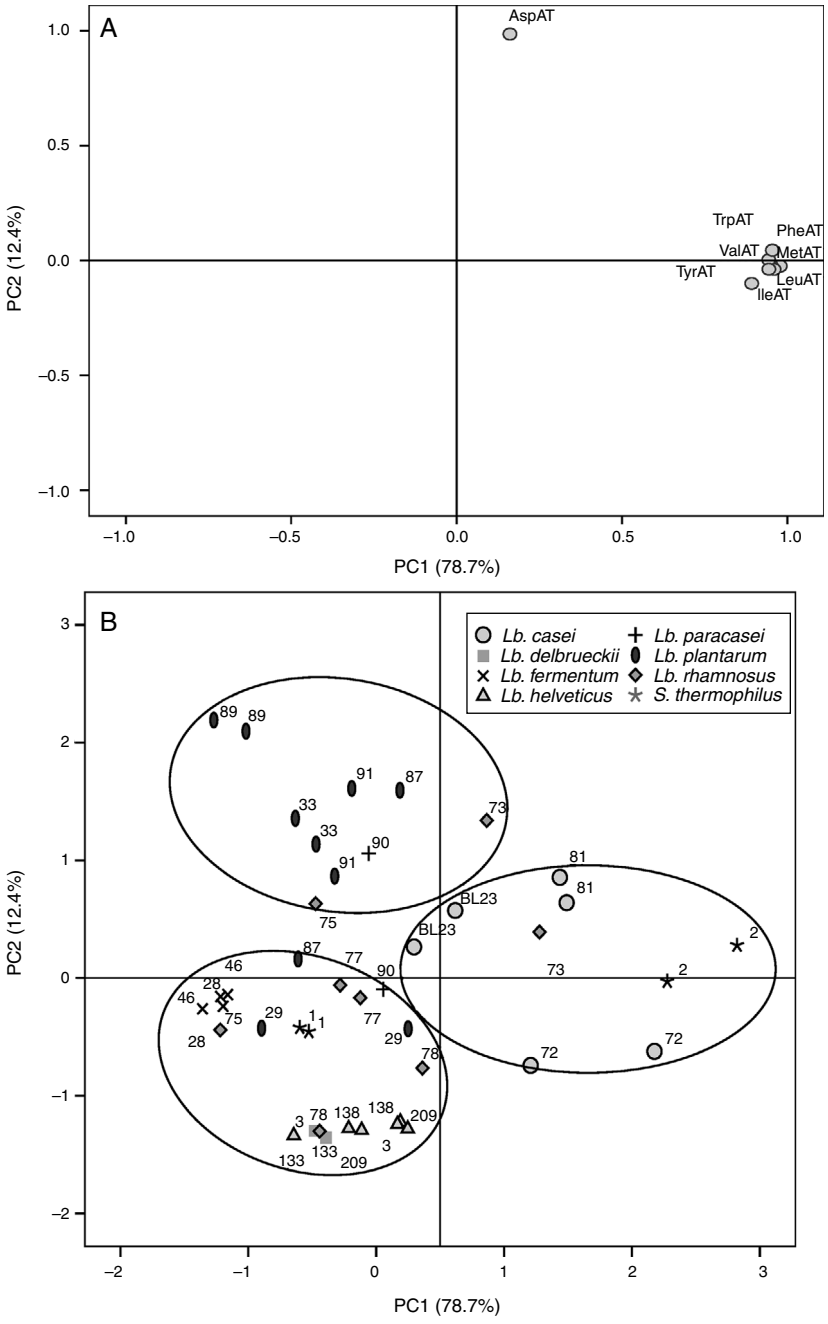

Fig. 2 – Principal component analysis (PCA) of AT activities toward eight AAs for the 21 strains: A – Loading plot (PC1 vs. PC2), B – Score plot (PC1 vs. PC2). Ellipses enclose samples grouped according to hierarchical cluster analysis (HCA).

the four strains of thermophilic lactobacilli and *L. rhamnosus* 78 were characterized by the lowest levels of AT for Asp.

In agreement with our results, other researchers have reported high Asp-AT activities in *L. paracasei* and *L. plantarum* strains.[8,19] This AT profile is interesting for adjunct cultures when diacetyl and acetoin are the desired flavor compounds produced via an alternative or complementary metabolic pathway for the catabolism of citrate by citrate-positive strains.[2,30] Diacetyl and acetoin formation from Asp has been demonstrated in reaction mixtures with α-ketoglutarate,[30] as well as in cheese models.[19,31]

On the other hand, while screening cheese-related cultures for AT activity, Smit et al.[32] found that Leu-AT predominated in *Lactococcus* strains, followed by *S. thermophilus* and *L. acidophilus*; the lowest levels were found in *L. casei*, *L. helveticus*, and *Propionibacterium*, *Leuconostoc*, and *Bifidobacterium* spp. Also, Kieronczyk et al.[19] reported higher levels of AT activity against branched-chain AAs in *Lactococcus lactis* compared with *L. paracasei*. In the present work, the highest levels of Leu-AT were found in *S. thermophilus* 2, followed by the *L. casei* strains, while the lowest levels were revealed by the *L. fermentum* strains.

Table 3 – Endogenous aminotransferase activity in CFE of the 21 tested strains.

Species	Strain	Endogenous AT activity
L. plantarum	33	0.67 ± 0.07
	91	1.23 ± 0.04
	89	0.03 ± 0.03
	29	1.20 ± 0.04
	87	0.79 ± 0.38
L. casei	81	1.51 ± 0.03
	72	2.18 ± 0.55
	BL 23	0.93 ± 0.17
L. paracasei	90	1.13 ± 0.04
L. rhamnosus	73	1.19 ± 0.15
	77	0.82 ± 0.05
	78	0.84 ± 0.17
	75	0.45 ± 0.33
L. fermentum	28	0.29 ± 0.01
	46	0.20 ± 0.01
L. delbrueckii	133	0.83 ± 0.08
L. helveticus	138	1.27 ± 0.27
	209	1.11 ± 0.14
	3	0.84 ± 0.19
S. thermophilus	1	0.60 ± 0.09
	2	2.46 ± 0.32

Activities are expressed as μg of glutamic produced from α-ketoglutarate per min and mg protein. Values are the means ± standard deviation of the results of two independent experiments.

Thermophilic lactobacilli cultures have been less characterized for their AT profiles than other lactobacilli. Jensen and Ardö[26] reported AT specificity toward aromatic and branched-chain AAs in six strains of *L. helveticus*, while Asp-AT was very low or undetectable. We confirmed the same trend for Asp-AT, but the other ATs showed equally low levels in the four thermophilic lactobacilli strains tested.

Even though no AT specific for Met has yet been isolated, activity toward this AA can be quantified in lactic cultures, and it is crucial for the production of important volatile compounds such as dimethyl disulfide, dimethyl trisulfide, methanethial, and methanethiol.[33] Among our strains, transformation of Met was mainly evidenced for *S. thermophilus* 2, the three strains of *L. casei*, and *L. rhamnosus* 73, while the lowest levels were found in the two strains of *L. fermentum*. Hanniffy et al.[18] found high levels of AT activity for Met in all *Lactococcus* strains studied, while lower or undetectable levels were found in strains of *L. casei*, *L. plantarum*, and *L. fermentum*. Other researchers have also reported this activity in strains of *L. casei* and *L. plantarum*,[3] as well as in *Lactococcus* strains.[34] Met metabolism is probably a result of non-specific activity of other ATs, such as Ar-AT or Bc-AT, as overlapping has been suggested.[7]

Finally, all strains showed endogenous AT activity ranging from a minimal level of 0.03 for *L. plantarum* 89 to levels higher than 2.0 for *S. thermophilus* 2 and *L. casei* 72 (Table 3). For some strains, including *L. plantarum* 29 and 91 and *L. fermentum* 28 and 46, this activity was relevant as it was at the levels of Ar-AT, Bc-AT, and Met-AT. However, even for these strains, the Asp-AT activity was always much higher than the endogenous AT activity. Endogenous AT activity has been explained by a high intracellular pool of Glu or non-specific transamination of diverse AAs in CFEs.[29] Williams et al.[29] detected this activity in 60% of the 29 strains of lactococci and mesophilic lactobacilli; in some cases, the level of the endogenous activity was comparable to that of Ar-AT or Met-AT.

Conclusions

Knowledge of GDH activity and AT profiles may be useful as selection criteria for starter and adjunct cultures in order to enhance or diversify cheese flavors. In the present work, we identified several strains of potential interest as flavor-forming cultures. Thus, both *S. thermophilus* strains showed the potential of producing α-ketoglutarate from glutamic acid due to their high GDH activity. As for the AT diversity, most mesophilic lactobacilli seem appropriate to promote the production of Asp-related flavor compounds, especially *L. plantarum* strains 89, 33, and 91, which may be proposed as adjunct cultures for this purpose. Branched-chain amino acid- and aromatic amino acid-derived compounds are also expectable from the use of *L. casei* 81, 72, and BL23, *L. rhamnosus* 73, and *S. thermophilus* 2, based on their profiles and levels of ATs.

Conflicts of interest

The authors declare no conflicts of interest.

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