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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