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Esterase Activities and Biochemical Properties of Lactic Acid Bacteria Isolated from Goat's Milk Cheese in Argentina

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Abstract: Twenty-two lactic acid bacteria (LAB) strains isolated from Argentinean goat dairy products were evaluated for its biochemical properties and esterase activities relevant to flavor development. *Streptococcus thermophilus* (UNSE314), *Lactobacillus* (L.) *delbrueckii* subsp. *bulgaricus* (UNSE309), *L. rhamnosus* (UNSE308), *L. plantarum* (UNSE287, UNSE316, UNSE317) and *Pediococcus pentosaceus* (UNSE315) strains presented high acidifying activity. All strains tested metabolized citrate and produced diacetyl-acetoin in goat milk. Based on these results, ten strains with the best performance in diverse technological properties were selected to determine esterolytic activity. In all evaluated strains, esterase specific activity (ESA) was detected on α-naphthyl (α-NA) acetate and β-naphthyl (β-NA) acetate, propionate, caprylate and α-NA butyrate. No activity was detected on β-NA laurate. The highest values were detected when using α-NA instead of β-NA derivatives as substrate. In *Pediococcus* strains, wide variability in ESA were observed, which were species- and strain-specific. These results allow us to select strains with biochemical properties and esterase activities to design starter and adjunct cultures that contribute to flavor development during cheese ripening, thus preserving the typical organoleptic characteristics of Argentinean goat cheeses.

Key words: Esterase activity, *Pediococcus*, autochthonous starter cultures, biochemical properties, goat's milk cheese.

1. Introduction

In Argentina, goat's milk is mostly processed into cheeses in the Northwest and central regions [1]. The cheese produced in Santiago del Estero (Northwest region) is made from raw goat's milk, without addition of starter or adjunct culture and plays an important role in the economy of the region [2]. Many farmers manufacture fresh cheeses using artisanal procedures so its quality is very variable, which makes its diffusion difficult in the international and national markets. A technology for the manufacture of such cheeses has not been appropriately developed due to absence of suitable starter or adjunct cultures. Lactic acid bacteria (LAB) are used as starter and adjunct cultures or are present as secondary microbiota in cheese fermentation [3]. The design of cultures with enzymatic activities and technological properties adapted to the physicochemical composition of milk and ripening conditions (temperature and humidity of the region) is necessary for artisanal cheese manufacture. Therefore, attention is focused on the

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isolation and characterization of LAB from the native microbiota of goat's milk and cheeses that could be used successfully in dairy industry.

Autochthonous LAB isolated from artisanal goat's milk cheeses from the Province of Santiago del Estero, Argentina were identified as 38% *Lactobacillus* (*L.*) and 62% cocci. The species isolated were *L. plantarum*, *L. fermentum*, *L. rhamnosus*, *L. casei*, *L. brevis*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *Enterococcus* (*E.*) *faecium*, *E. faecalis*, *Leuconostoc*, *Streptococcus thermophilus* and *Pediococcus* [4].

LAB used as commercial starter and adjunct cultures are selected by their biochemical properties such as acidification, proteolytic, esterolytic and lipolytic activities, contributing to the development of texture and organoleptic characteristics of cheeses (flavour) [5]. Cheese flavour is the result of glycolysis, proteolysis, lipolysis and citrate metabolism carried out by cheese microbiota [6].

The contribution of cheese microbiota to lipolysis occurs via the esterase/lipase systems [6]. Esterases from starter and nonstarter lactic acid bacteria (NSLAB) are responsible for the release of short-chain fatty acids (SCFA) from milk fat at elevated water activity (aw) and the synthesis of fruity flavor in dairy products [7]. However, there is little information about the contribution of esterases from LAB to the formation of flavor in cheese manufactured with goat milk [6], and mainly in the genus *Pediococcus* [8].

The aim of this study was to evaluate the technological properties and esterase activities of 22 LAB strains isolated from goat dairy products, in order to select the strains to be used as starter or adjunct cultures for the manufacture of Argentinean goat's milk cheese.

2. Materials and Methods

2.1 Microorganisms

Autochthonous strains provided by the Universidad Nacional de Santiago de Estero (UNSE), Argentina, were isolated from goat milk and cheeses [4]. In this study the biochemical properties and esterase activity of 22 strains belonging to the genera *Lactobacillus*, *Pediococcus* and *Streptococcus*, were evaluated (Tables 1 and 2).

All strains were grown in MRS broth (Biomerieux, Marcy L'Etoile, France) (De Man et al., 1960) for 16 h. *P. pentosaceus* and *L. plantarum* were incubated at 30 °C and 35 °C, respectively. *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* were incubated at 42 °C.

2.2 Biochemical Properties

2.2.1 Acidification Ability

Acidification ability was determined by measurements of titratable acidity, acidification rate and end pH in goat milk [4, 9]. Strains were inoculated to 2% (v/v) in 10% (w/v) reconstituted goat milk powder and incubated at 35 °C by 16 h. Titratable acidity was determined by titration with 0.11 N NaOH in the presence of phenolphthalein and was expressed as g/L of lactic acid.

The end pH was determined with pHmetre (Ω Metrohom pHmetro 692, Herisau, Switzerland). Acidification rate of strains was expressed as ΔpH/min, ΔpH = pH end – pH initial (ΔpH/min) [10].

To distinguish between fast- and slow-growing cultures, the capacity to coagulate milk and the type of growth in fast-slow differentiation agar [11] were determined.

2.2.3 Citrate Utilization

Citrate utilization [12] was considered positive when clearing zones around colonies (> 3 mm) on calcium citrate medium were observed.

2.2.4 Diacetyl and Acetoin Production

Diacetyl-acetoin production in goat milk was determined according to Medina et al. [13]. Strains were inoculated to 2% (v/v) in 10% (w/v) reconstituted goat milk powder and incubated at 35 °C, 16 h.

Table 1 Biochemical properties of *Lactobacillus* isolated from goat's dairy products.

Strains	Acidification ability[a]				Citrate utilization[f]	DA[g]
	Titratable acidity[b]	pH end[c]	Acidification rate[d]	FSDA[e]		
L. plantarum						
UNSE53	8.40 ± 0.10^{BCD}	5.80 ± 0.02^{BCD}	0.0007 ± 0.0003^{D}	Fast	+	+
UNSE186	7.80 ± 0.10^{E}	5.82 ± 0.02^{BC}	0.0007 ± 0.0003^{D}	Fast	+	+
UNSE206	6.80 ± 0.10^{FG}	5.74 ± 0.02^{CDE}	0.0008 ± 0.0003^{CD}	Fast	+	+
UNSE213	5.00 ± 0.20^{I}	5.58 ± 0.02^{FG}	0.0010 ± 0.0003^{BCD}	Slow	+	+
UNSE218	7.20 ± 0.10^{F}	5.55 ± 0.02^{G}	0.0010 ± 0.0003^{BCD}	Fast	+	+
UNSE243	4.70 ± 0.20^{I}	5.73 ± 0.01^{DE}	0.0008 ± 0.0002^{CD}	Slow	+	+
UNSE287	8.70 ± 0.10^{BC}	5.30 ± 0.02^{H}	0.0014 ± 0.0003^{AB}	Fast	+	+
UNSE300	5.90 ± 0.20^{H}	5.96 ± 0.02^{A}	0.0006 ± 0.0003^{D}	Fast	+	+
UNSE301	6.40 ± 0.20^{GH}	5.85 ± 0.01^{B}	0.0007 ± 0.0002^{D}	Fast	+	+
UNSE316	9.40 ± 0.20^{A}	4.90 ± 0.02^{I}	0.0017 ± 0.0003^{A}	Fast	+	+
UNSE317	9.20 ± 0.10^{AB}	5.20 ± 0.02^{H}	0.0014 ± 0.0003^{AB}	Fast	+	+
L. rhamnosus						
UNSE308	8.20 ± 0.10^{CDE}	5.30 ± 0.02^{H}	0.0014 ± 0.0003^{AB}	Fast	+	+
L. delbrueckii subsp. *bulgaricus*						
UNSE309	8.00 ± 0.20^{DE}	5.20 ± 0.02^{H}	0.0013 ± 0.0003^{ABC}	Fast	+	+
UNSE313	8.40 ± 0.02^{BCD}	5.70 ± 0.01^{E}	0.0008 ± 0.0001^{CD}	Fast	+	+

[a]Mean value ± SD; [b]titratable acidity (g/L of lactic acid) in milk, 16 h, 35 °C; [c]pH end of goat milk determined at 16 h, 35 °C; [d]acidification rate (absolute value): ΔpH/min, ΔpH = pH end – pH initial; [e]fast-slow differentiation agar (FSDA); [f]calcium citrate medium: +, zones of clearing around colonies; -, colonies without zones of clearing; [g]diacetyl-acetoin (DA): +, production; -, absence of production; [A-I]values in the same column with different superscript letters differ significantly ($P < 0.05$).

Table 2 Biochemical properties of *S. thermophilus* and *P. pentosaceus* isolated from goat's dairy products.

Strains	Acidification ability[a]				Citrate utilization[f]	DA[g]
	Titratable acidity[b]	pH end[c]	Acidification rate[d]	FSDA[e]		
S. thermophilus						
UNSE314	9.20 ± 0.10^{A}	4.40 ± 0.02^{E}	0.0022 ± 0.0003^{A}	Fast	+	+
P. pentosaseus						
UNSE22	6.10 ± 0.10^{CD}	5.80 ± 0.02^{B}	0.0007 ± 0.0003^{B}	Slow	+	+
UNSE216	6.20 ± 0.10^{CD}	5.76 ± 0.01^{B}	0.0008 ± 0.0002^{B}	Slow	+	+
UNSE253	5.00 ± 0.20^{E}	6.00 ± 0.02^{A}	0.0006 ± 0.0003^{B}	Slow	+	+
UNSE310	6.40 ± 0.10^{BC}	5.60 ± 0.02^{C}	0.0009 ± 0.0003^{B}	Fast	+	+
UNSE311	6.60 ± 0.20^{B}	5.40 ± 0.02^{D}	0.0011 ± 0.0003^{B}	Fast	+	+
UNSE312	6.00 ± 0.20^{D}	5.80 ± 0.01^{B}	0.0007 ± 0.0002^{B}	Fast	+	+
UNSE315	9.00 ± 0.10^{A}	4.50 ± 0.02^{E}	0.0021 ± 0.0003^{A}	Fast	+	+

[a]Mean value ± SD; [b]titratable acidity (g/L of lactic acid) in milk, 16 h, 35 °C; [c]pH end of goat milk determined at 16 h , 35 °C; [d]Acidification rate (absolute value): ΔpH/min, ΔpH = pH end – pH initial; [e]fast-slow differentiation agar (FSDA); [f]calcium citrate medium: +, zones of clearing around colonies; -, colonies without zones of clearing, [g]diacetyl-acetoin (DA): +, production; -, absence of production; [A-E]values in the same column with different superscript letters differ significantly ($P < 0.05$).

2.3 Preparation of Cell Free Extracts

Cells cultured in 400 mL of MRS broth were harvested after 16 h by centrifugation at 10,000 × g for 10 min at 4 °C, washed twice with 50 mM sodium phosphate buffer, pH 7.0 and resuspended in 500 g/L sodium phosphate buffer. The suspension was disrupted by adding 1 g to 2 g of glass beads (Nº 31/14: diameter, 0.10-0.11 mm, B. Braun Biotech International, Germany) and using a cell disruptor (B. Braun Melsungen AG, Germany). Five mixing sequences (speed, 6.5 /s) of each 1 min were

successively applied under CO_2 atmosphere. Samples were cooled on ice for 1 min between each mixing sequence. Cellular debris was removed by centrifugation (20,000 × g for 30 min at 4 °C) and supernatant was used as cell free extract (CFE) by esterase activity determination.

2.4 Esterase Activity

Esterase activity of CFE was determined on α-naphthyl (α-NA) derivatives (C2, C3, C4, C8, C10 and C12 of carbon atoms) and β-naphthyl (β-NA) derivatives (C2, C3, C4, C8 and C12 carbon atoms) as substrates (Sigma-Aldrich, St. Louis, Missouri, USA) [14]. The assay mixture contained 88 μL of 100 mM sodium phosphate buffer (pH 7.00), 2 μL of α-NA and β-NA substrate (10 mM in ethanol) and 10 μL of CFE. After incubation for 30 min at 35 °C, the color was developed by adding 60 μL of Fast Garnet GBC (Sigma-Aldrich) preparation (5 g/L in 100 g/L sodium dodecyl sulfate (SDS)) and further incubation at room temperature for 15 min. The absorbance was measured by using tunable microplate reader (Versamax TM, Molecular Devices, Sunnyvale, California, USA) 560 nm. Two standard curves were prepared using α-naphthol or β-naphthol.

A unit of esterase activity was defined as the amount of enzyme that released 1 μmol α-naphthol/min or 1 μmol β-naphthol/min. Esterase specific activity (ESA) was defined as units per milligram of protein (U/mg).

2.5 Protein Determination

Concentrations of proteins in CFE were determined by the method of Bradford [15] with bovine serum albumin (Sigma) as the standard.

2.6 Statistical Analysis

ANOVA analysis (InfoStat 2011, Grupo Infostat, FCA, Universidad Nacional de Córdoba, República Argentina) was carried out to determine statistical differences ($P \leq 0.05$) between the strains of a single bacterial species with respect to the values of acidification (in milk and curd) and esterase activity. Tukey test was used ($\alpha \leq 0.05$). All experiments were repeated twice.

3. Results and Discussion

3.1 Biochemical Properties

Acidification activity is an important parameter in the selection of starter strains [13]. Studied strains were classified into three groups according to the level of acidification reached: low (≤ 5 g/L lactic acid), medium (between 5 g/L and 7 g/L lactic acid) and high (≥ 7 g/L lactic acid) as shown in Tables 1 and 2 for *Lactobacillus* and cocci, respectively.

Three categories of acidification ability were detected in *L. plantarum* strains, high (54.54%), medium (27.27%) and low (18.18%). Xanthopoulos et al. [16] reported different acidification ability in *L. plantarum* strains isolated from dairy origin. *L. delbrueckii* subsp. *bulgaricus* UNSE309 and *L. rhamnosus* UNSE308 presented high acidification ability and similar results were obtained by Cheriguene et al. [17].

Thermophilic LAB strain (*S. thermophilus* UNSE314) presented high acidification ability as would be expected for homofermentative species.

Strains with high acidification ability play an important role in the initial milk coagulation process and prevention or reduction of adventitious microbiota growth [18] so they can be used as starter culture. Starter cultures are composed by a limited number of thermophilic bacteria such as, *Streptococcus thermophilus, L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, *L. helveticus, L. plantarum* and mesophilic LAB such as *L. paracasei, Lactococcus lactis* and *Leuconostoc mesenteroides* ssp. *cremoris* [19].

Regarding to *P. pentosaceus* strains assayed, *P. pentosaceus* UNSE315 presented high acidifying ability similar to *S. thermophilus* UNSE314. However, 71% of pediococci presented medium acidification ability. Similar results were found by Ayad et al. [10]

for pediococci isolated from artisanal cheese. Strains with slow acidification ability can be used as adjunct cultures depending on their other important properties, e.g., diacetyl production and esterase activities.

Highest acidification rate values were detected in *L. plantarum* UNSE316, UNSE287 and UNSE317, *L. rhamnosus* UNSE308, *L. delbrueckii* subsp. *bulgaricus* UNSE309, *S. thermophilus* UNSE314, and *P. pentosaceus* UNSE315.

Fast acid production in fast-slow differentiation agar (FSDA) was detected in 77.3 % of studied strains. Similar results were reported by Oliszewski et al. [20] in LAB strains isolated from goat's dairy products. *L. plantarum* UNSE243 and UNSE213 showed slow acid production in FSDA medium.

In general, differences in acidification ability values were found between strains, even within the same species. These are in agreement with those reported by Oliszewski et al. [20] and Nieto-Arribas et al. [21].

All studied strains utilized citrate in agar medium and produced diacetyl and acetoin in goat milk (Tables 1 and 2). Citrate utilization is an important technological characteristic of some starter LAB. Citrate in milk is metabolized by many species of LAB into flavour compounds, such as diacetyl, acetoin, and 2, 3-butanediol [22]. The strains which are able to ferment citrate that is naturally present in milk and thus produce aromatic substances such as diacetyl, confer the typical butter aroma [23].

NSLAB constitute the predominant cheese microbiota during ripening and strongly influence flavor development and the final characteristics of cheese [8]. The NSLAB strains used as adjunct cultures are mainly mesophilic lactobacilli especially *L. paracasei* subsp. *paracasei* and *L. plantarum* and pedicocci such as *Pediococcus* (*P.*) *pentosaceus* and *P. acidilactici* and *Enterococci* spp. [24, 25]. Enhanced flavour production in brined cheeses such as Feta and Teleme was observed with the incorporation of *P. pentosaceus* as adjunct culture [25].

3.2 Esterase Activity

ESA was evaluated in LAB strains: UNSE287, UNSE308, UNSE309, UNSE314, UNSE315, UNSE316 and UNSE317 with high acidification ability were used as potential starters cultures; UNSE22, UNSE216, UNSE253 with low acidification ability were used as adjunct culture (Tables 3 and 4).

Table 3 Specific esterase activity[a] in cell free extract of lactic acid bacteria isolated from goat's dairy products.

Source of enzyme	Substrate α-naphthyl derivative of fatty acid					
	Acetate (C2)	Propionate (C3)	Butyrate (C4)	Caprylate (C8)	Caprate (C10)	Laurate (C12)
L. rhamnosus						
UNSE 308	5.10 ± 0.33^{D}	2.30 ± 0.12^{E}	1.20 ± 0.15^{F}	1.10 ± 0.12D	N.D.	N.D.
L. bulgaricus						
UNSE309	14.80 ± 0.63^{B}	9.90 ± 0.44^{B}	2.70 ± 0.18^{E}	1.30 ± 0.14^{D}	N.D.	N.D.
L. plantarum						
UNSE287	25.70 ± 0.52^{A}	11.20 ± 0.54^{A}	2.50 ± 0.20^{E}	1.40 ± 0.12^{D}	N.D.	N.D.
UNSE316	24.60 ± 0.60^{AB}	10.90 ± 0.45^{AB}	1.90 ± 0.12^{F}	2.40 ± 0.16^{C}	N.D.	N.D.
UNSE317	7.30 ± 0.56^{CD}	6.60 ± 0.32^{DE}	6.40 ± 0.28^{C}	7.20 ± 0.43^{A}	3.60 ± 0.20^{A}	N.D.
S. thermophilus						
UNSE314	2.60 ± 0.32^{E}	1.60 ± 0.22^{E}	1.90 ± 0.12^{F}	1.20 ± 0.12^{D}	N.D.	N.D.
P. pentosaceus						
UNSE22	2.40 ± 0.23^{E}	7.70 ± 0.40^{C}	8.20 ± 0.42^{B}	2.60 ± 0.14^{C}	3.00 ± 0.20^{A}	N.D.
UNSE216	2.20 ± 0.21^{E}	6.40 ± 0.34^{CD}	10.20 ± 0.54^{A}	1.40 ± 0.12^{D}	N.D.	N.D.
UNSE253	8.40 ± 0.23^{C}	8.20 ± 0.28^{BC}	4.50 ± 0.33^{D}	1.20 ± 0.10^{D}	1.90 ± 0.14^{B}	N.D.
UNSE315	2.20 ± 0.21^{E}	6.40 ± 0.25^{CD}	1.20 ± 0.14^{F}	5.00 ± 0.30^{B}	1.70 ± 0.12^{B}	1.60 ± 0.12^{A}

[a]Specific esterase activity was expressed as units per milligram of protein. Mean ± standard deviation. [A-F]Values corresponding to different bacterial strains of the same column not showing a common superscript differ significantly ($P < 0.05$). N.D.: not detected.

Table 4 Specific esterase activity[a] in cell free extract of lactic acid bacteria isolated from goat's dairy products.

Source of enzyme	Substrate β-naphthyl derivative of fatty acid					
	Acetate (C2)	Propionate (C3)	Butyrate (C4)	Caprylate (C8)	Caprate (C10)	Laurate (C12)
L. rhamnosus						
UNSE 308	6.80 ± 0.21^{C}	8.40 ± 0.32^{B}	2.30 ± 0.14^{C}	0.70 ± 0.05^{E}	N.D.	N.D.
L. bulgaricus						
UNSE309	11.90 ± 0.32^{A}	8.70 ± 0.28^{B}	1.90 ± 0.12^{CD}	5.50 ± 0.25^{B}	N.D.	N.D.
L. plantarum						
UNSE287	8.20 ± 0.22^{B}	10.30 ± 0.32^{A}	10.40 ± 0.32^{AB}	7.80 ± 0.34^{A}	N.D.	N.D.
UNSE316	3.40 ± 0.20^{DE}	0.80 ± 0.05^{E}	1.20 ± 0.10^{D}	1.50 ± 0.10^{D}	N.D.	N.D.
UNSE317	4.10 ± 0.24^{D}	4.60 ± 0.22^{C}	3.80 ± 0.22^{B}	5.50 ± 0.23^{B}	N.D.	N.D.
S.thermophilus						
UNSE314	5.60 ± 0.23^{CD}	1.30 ± 0.12^{D}	1.50 ± 0.16^{D}	0.80 ± 0.04^{E}	N.D.	N.D.
P.pentosaceus						
UNSE22	1.20 ± 0.23^{F}	3.60 ± 0.14^{CD}	N.D.	5.00 ± 0.21^{B}	N.D.	N.D.
UNSE216	1.40 ± 0.20^{F}	0.60 ± 0.07^{E}	N.D.	1.60 ± 0.06^{D}	N.D.	N.D.
UNSE253	1.80 ± 0.12^{F}	3.60 ± 0.22^{CD}	N.D.	2.30 ± 0.24^{C}	N.D.	N.D.
UNSE315	2.10 ± 0.14^{E}	4.80 ± 0.24^{C}	11.30 ± 0.34^{A}	2.30 ± 0.20^{C}	N.D.	N.D.

[a]Specific esterase activity was expressed as units per milligram of protein. Mean ± standard deviation. [A-F]Values corresponding to different bacterial strains of the same column not showing a common superscript differ significantly ($P < 0.05$). N.D.: not detected.

ESA was detected on α-NA C2, C3, C4 and C8 carbon atoms in all studied strains, as shown in Table 3. When ESA was evaluated on α-NA caprate, activity was detected in *L. plantarum* UNSE317 and *P. pentosaceus* UNSE22, UNSE253 and UNSE315 strains. *P. pentosaceus* UNSE315 was the only strain with activity on α-NA laurate.

Regarding to lactobacilli strains, high activities were observed in *L. plantarum* UNSE287 on α-NA acetate and propionate (25.70 U/mg and 11.20 U/mg, respectively). *L. plantarum* UNSE316 showed high ESA on α-NA acetate and propionate (24.60 U/mg and 10.90 U/mg, respectively). *L. delbrueckii* subsp. *bulgaricus* UNSE309 showed the highest ESA on α-NA acetate (14.80 U/mg) and the lowest ESA on α-NA caprylate (1.30 U/mg), and these results are in agreement with Oliszewski et al. [26]. *L. rhamnosus* UNSE308 presented the highest activity on α-NA acetate and hydrolyzed α-NA derivatives of fatty acids of three, four and eight carbon atoms as substrates.

Regarding to cocci, in *S. thermophilus* UNSE314, the highest ESA was observed on α-NA acetate (2.60 U/mg). In pediococci, the highest activities were observed on α-NA acetate and propionate (8.40 U/mg, 8.20 U/mg, respectively) in *P. pentosaceus* UNSE253. High activities were observed on α-NA butyrate, caprylate and caprate in *P. pentosaceus* UNSE216, UNSE315 and UNSE22, respectively. Oliszewski et al. [26] reported that one strain *Pedicoccus pentosaseus* only had esterase activity on a-NA acetate.

When β-NA of fatty acids was used as substrate, ESA was detected on β-NA C2, C3 and C8 carbon atoms in all studied strains (Table 4). No activity was observed on β-NA laurate. The highest activities were detected in *L. delbrueckii* subsp. *bulgaricus* UNSE309 on β-NA acetate and propionate, in *L. plantarum* UNSE287 on acetate, propionate, butyrate and caprilate, and in *L. rhamnosus* UNSE308 on β-NA acetate and propionate.

Regarding to cocci strains, the highest activity was observed in *P. pentosaceus* UNSE315 on β-NA butyrate (11.30 U/mg). Vafopoulou-Mastrojiannaki et al. [27] reported that *P. pentosaceus* isolated from traditional Greek cheeses was detected a low esterase activity using 4-nitrophenyl butyrate as substrate. In our work, significant activities were detected for *P. pentosaceus* UNSE22 on β-NA propionate and caprylate, *P. pentosaceus* UNSE253 on β-NA

propionate and *S. thermophilus* UNSE314 on β-NA acetate.

To date, there is little information about the esterase activity in pediococci strains of dairy origin [26, 27].

Among the strains under study, the highest values were detected when using α-NA instead of β-NA derivates as the substrate. These results indicate that esterase enzymes are dependent on the positional isomeric of the fatty acid derivative used as substrate. LAB strains evaluated in this work presented wide variations in ESA, which were species- and strain-specific. Similar results were reported by Gobetti et al. [28] and Katz et al. [29].

The preference of esterase LAB to release SCFA from esters accords with results reported by other authors [29, 30]. SCFA such as acetic, butanoic and hexanoic acids are potent cheese flavour compounds [31, 32]. Esterases of LAB evaluated in this study released C2-C8 fatty acids. Esterases have the capacity for both hydrolyzing and synthesizing esters by esterification of fatty acids and ethanol [7]. These ethyl esters, such as ethyl butanoate and ethyl hexanoate (at less than 5 ppm) are believed to play an important role in the development of characteristic fruity flavors in some varieties of cheeses [31, 33].

4. Conclusions

The strains evaluated in this study exhibited biochemical characteristics and esterase activities, which could be responsible for the development of flavor found in Argentinean goat's milk cheeses. The use of autochthonous starter or adjunct cultures with evaluated technological properties ensures the obtaining of cheeses with constant organoleptic quality preserving the identity of traditional cheeses. Our results provide the basis for the selection of the following strains: *L. delbrueckii* subsp. *bulgaricus* UNSE309 and *S. thermophilus* UNSE314 because of their desirable acidifying capacity and specific esterase activities. *L. rhamnosus* UNSE308 and *L. plantarum* UNSE287, UNSE316 and UNSE317 strains could be used as starter or adjunct culture to complement the activities present in the starter and influence flavor development during cheese ripening. *P. pentosaceus* UNSE22, UNSE216 and UNSE253 would be included as an adjunct culture for their specific esterase activities and contribution on flavor development.

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