Technological properties and biogenic amines production by bacteriocinogenic lactococci and enterococci strains isolated from raw goat's milk

Running head: Technological and biogenic amines of lactococci and enterococci

Technological Properties and Biogenic Amines Production by Bacteriocinogenic Lactococci and Enterococci Strains Isolated from Raw Goat Milk

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ABSTRACT

Technological properties and biogenic amine (BA) production were analyzed in fifty-six bacteriocinogenic lactococci and enterococci strains isolated from raw goat milk. Fifteen lactococci strains were able to reduce milk pH to 5.3 or lower, after 6 h while enterococci strains were initially slow in producing acids. *L. lactis* subsp. *lactis* GLc06 and three *E. faecalis* (GEn20, GEn22, and GEn23) presented high proteolytic activity. GLc06 and *E. faecalis* GEn22, also showed a high percentage of autolysis already after 4 h, reaching to 71.11 % and 97.67 % after 24 h, respectively. No strain was able to secrete EPS and *L. lactis* subsp. *lactis* GLc22 and 25 *Enterococcus* were able to produce diacetyl. *L. lactis* subsp. *lactis* GLc05 and other 23 *Enterococcus* strains presented a high tolerance to NaCl at 10% (w/v). Considering the BA production, 12 strains (5 lactococci and 7 enterococci) were capable to form tyramine and 4 strains (1 lactococcus and 3 enterococci) were capable to form 2-phenylethylamine, but in very low amounts. GLc06 presented a great acidifying, proteolytic and autolytic activity and GLc05 was capable to grow at high NaCl concentrations (10 % w/v), possessing a medium autolytic and proteolytic activity. Some enterococci strains presented the ability to produce diacetyl and a high autolytic and extracellular proteolytic activity and also presented resistance to high NaCl concentrations. The interesting technological properties presented by some bacteriocinogenic strains can justify their use by the dairy industry, aiming both safety due to bacteriocin production and technological transformations in fermented products.

The microbiota found in fermented foods such as raw milk cheeses is derived mainly from raw material by direct or indirect transfer of autochthonous microbiota *(22)*. Lactic acid bacteria (LAB) is the main microbial group found in raw goat milk and have a very important role in fermented foods due to its technological properties and biopreservative potential *(30, 31)*.

Due to competition with other microorganisms, wild strains often produce antimicrobial substances, such as bacteriocins, that inhibit the development of pathogens and spoilage microorganisms enhancing the safety and shelf-life of final products *(20, 27, 30)*. The use of bacteriocinogenic wild strains of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Enterococcus* as acidifying and/or flavouring starters in cheese production is very promising *(39)*.

Specific sensory characteristics are generated from LAB metabolic pathways resulting in a diversity of aromatic compounds *(22)*. They convert the sugar into lactic acid resulting in rapid acidification of raw material and also produce other metabolites such as ethanol, diacetyl, acetate, acetaldehyde, etc, that improves the flavor, texture, taste, storage and safety of the end products *(20)*. The production of proteases and intracellular peptidases allows the hydrolyze of casein and peptidases into small peptides and free amino acids, essential in the cheese ripening *(1, 33)*. LAB autolysis allows the intracellular peptidases to reach their substrates and accelerate the cheese ripening *(33)*. Also, some strains are tolerant to diverse values of pH, salt concentration (6 and 10% NaCl/water), and a wide range of temperature (2 - 53 °C).

Obviously, the selection of potential starter cultures must not only be focus on their functional properties, but also on the production of undesirable factors, such as the production of biogenic amines (BA). Some LAB present in the food matrix, mainly enterococci, can produce decarboxylases that convert free amino acids into BA, especially tyramine, 2-phenylmethylamine, tryptamine, cadaverine, putrescine and histamine. Quantification of BA production by LAB is essential, because only high amounts of BA can constitute a health risk to

the consumers.

In previous studies, bacteriocinogenic LAB strains were isolated from raw goat milk and their antimicrobial and virulence potential were properly characterized *(29, 30)*. Based on these previous studies, the present study aimed to evaluate the technological potential of the isolated bacteriocinogenic lactococci and enterococci strains from raw goat milk, and also to quantify their production of BA.

MATERIAL AND METHODS

Strains and culture conditions. In the present study were used twenty tree lactococci (20 *L. lactis* subsp. *lactis* and 3 *L. lactis*) and 33 enterococci (17 *E. durans*, 8 *E. faecalis*, 7 *E. faecium* and 1 *E. hirae*). These strains were isolated from raw goat milk, characterized as bacteriocinogenic *(30)* and their safety potential were previously determined *(29)*. Stock cultures were kept frozen (-80 °C) in de Man Rogosa and Sharpe (MRS, Oxoid Ltd., Basingstoke, England) broth containing 20 % (v/v) glycerol.

Technological properties of bacteriocinogenic strains. For acidifying activity, aliquots (1% v/v) of overnight cultures growth in MRS (Oxoid) were inoculated in 10 mL of UHT goat milk and incubated for 24 h at 30 °C. pH values of the cultures were measured after 6 and 24 h of incubation, using pH meter (HI 221, Hanna Instruments, São Paulo, SP, Brazil). For exopolysaccharide (EPS) production, aliquots (1% v/v) of each overnight culture were inoculated in 10 mL of skim milk (10% w/v, Molico, Nestle, São Paulo, SP, Brazil) and incubated for 24 h at 30 °C. The EPS production from lactose was qualitatively determined by the degree of 'stringiness' of cultures. A positive result was recorded if the coagulated culture could be teased

into a string with an inoculating loop *(6)*. Exocellular proteolytic activity was assessed by using aliquots (1 µL) from overnight cultures spotted onto the surface of 10 % (w/v) skim milk (Molico) and 2 % (w/v) agar and incubated for 4 days at 30 °C *(13)*. The proteolytic activity was indicated as a clear halo around the colonies, the radius values were measured and presented in millimeters (mm). For diacetyl production, aliquots (1% v/v) from overnight cultures were inoculated in 10 mL of skim milk (10% p/v, Molico) and incubated for 24 h at 30 °C. Then, 1 mL of each sample was added with 0.5 mL of α-naphtol (1% w/v) and KOH (16% w/v) and incubated for 10 min at 30 °C. The diacetyl production was indicated by the formation of a red ring at the top of the tubes *(13)*. The resistance of the strains to different NaCl concentrations was evaluated in according to Dal Bello *et al. (9)*. Aliquots (100 µL) were inoculated in MRS broth with different NaCl concentrations (0, 4, 6 and 10%) and incubated for 24 h at 30 °C. At the times t = 0 h and t = 24 h, the bacterial growth was monitored by measuring the optical density at 650 nm using the BioMate 3S Spectrophotometer (Thermo Scientific Inc., San Jose, CA, USA) and the growth measurement was performed subtracting the OD value at t = 0h from OD value at t = 24h. Autolysis was measured according to Nieto-Arribas *et al. (25)*. The LAB strains were inoculated in MRS broth and incubated at 30 °C until they reached the OD_{650nm}= 0.8 to 1.0. Then, aliquots of 1 mL of the cultures were centrifuged (1,000 x *g* for 5 min at 4 °C) and the pellet was washed twice with K_3PO_4 (50Mm, pH=6.5). The optical density was measured at 0, 4 h and 24 h using the BioMate 3S Spectrophotometer (Thermo Scientific). The analyses were conducted in triplicate. The autolysis degree was determined as 100 – (A1/A2 x 100), where A1 is the lowest and A2 is the highest value of the OD_{650nm} measured during incubation.

Quantification of Biogenic Amines production by HPLC. Based on the phenotypical and molecular results for BA production *(29)*, fourteen strains (eight *Enterococcus* spp. and six

Lactococcus spp.) were selected in order to quantify the BA production by HPLC. Strains were grown in MRS broth (Oxoid) overnight, centrifuged (14,000 x *g* for 5 min), and the pellets were suspended in 1 mL of Ringer solution. The solution were transferred to 20 mL of skimmed milk (10 % w/v) and incubated at 37 °C for 24 h. The BA amounts were quantified after the extraction and derivatization steps as reported by Innocente *et al. (16)*, with modifications. BA quantification was performed using a Thermo-Finnigan Spectra System HPLC (Thermo Scientific) equipped with a P2000 binary gradient pump, a SCM 1000 degasser, an AS 3000 automatic injector, and a Finnigan Surveyor PDA Plus detector (PDA, Thermo Scientific). The ChromQuest software 5.0 (Thermo Scientific) was used for instrument control as well as for UV data collection and processing. Separation was achieved on a C18 RP Lichrosphere 250 × 4.6 mm, 5 μm (Merck Millipore, Darmstadt, Germany) column equipped with a C18 RP Lichrosphere guard column 5 μm (Merck Millipore). The following external standards were used: 2-phenylethylamine, putrescine, histamine, cadaverine, 1,7-diaminoheptane (IS), tyramine, and spermidine. All standards were of analytical grade and purchased from Sigma Aldrich (St. Louis, MI, USA). The results were expressed in mg/kg and the mean counts and standard deviation of tree repetitions were calculated.

RESULTS AND DISCUSSION

In the present study the acidifying activity of 23 lactococci and 33 enterococci bacteriocinogenic strains was assayed and the results presented in Tables 1 and 2, respectively. The rapid drop of pH is very important during cheese production, contributing for cheese texture and controlling undesirable microorganisms. According to Beresford *et al. (4)*, starter strains should be able to

produce sufficient acid to reduce the milk pH to 5.3 or lower after 6 h at 30 to 37 °C. Fifteen lactococci strains were able to reduce the milk pH to 5.3 or lower after 6 h (Table 1). Some studies have already demonstrated that most LAB strains are initially slow in producing acids *(9, 13, 23)*, such as the enterococci strains in the present work that presented high milk acidification only after 24 h (Table 2).

In general lactococci were able to reduce the pH faster than enterococci strains and acid production by enterococci was already classified as medium *(23)*. Rapid milk acidification by lactococci strains was also demonstrated by Picon *et al. (31)*, however Ribeiro *et al. (35)* found enterococci strains with higher acidification potential. According to Wouters *et al. (39)*, it is usual that wild lactococci are less acidifying than commercial strain. It may indicate that acid production by LAB is strain-dependent, which was already demonstrated in other studies *(25, 33)*.

The proteolytic activity of lactococci and enterococci strains were assayed by mensuration in millimeters of clear halos in the plates and the obtained results are present in Tables 1 and 2. One *L. lactis* subsp. *lactis* (GLc06) and tree *E. faecalis* (GEn20, GEn22, and GEn23) presented high proteolytic activity (Tables 1 and 2). Six *Enterococcus* (GEn19, GEn26, GEn29, GEn30, GEn31, and GEn32) and *L. lactis* subsp. *lactis* GLc21 showed no extracellular proteolytic activity (Tables 1 and 2). Proteolytic activity is an essential property for starter cultures; the proteolytic enzymes degrade caseins and peptides generating most of the aroma precursors, influencing the characteristics of "flavor" in yogurt and cheeses. Proteolytic enzymes plays a major role in the dairy products fermentation *(34)*. However, sometimes is preferable to not use strains with high proteolytic activity for production of some types of cheese, because excessive proteolysis can cause a high production of bitter peptides and other undesirable compounds or, excessive hydrolysis of casein can generate a final product too soft *(25)*. Among enterococci, *E. faecalis* is

considered as the most proteolytic specie *(15, 35, 37)*. However, until now the use of enterococci strains in cheese production is not permitted legally, because many strains possess pathogenic and toxigenic potential. Indeed, it is a controversial issue because some strains do not possess virulence factors, being able to produce bacteriocins and trigger beneficial transformations in fermented products. The idea of using enterococci in food must be carefully analyzed concerning the public health *(24)*.

The intact bacterial cells are necessary for physiological activities, such as lactose fermentation and oxygen removal, and for a number of flavor reactions. In contrast, the main consequence of autolysis in cheese is to accelerate the peptidolytic reactions. The percentage of autolysis after 4 h and 24 h presented by lactococci and enterococci strains are show in Tables 1 and 2, respectively. *L. lactis* subsp. *lactis* GLc06 and *E. faecalis* GEn22 that presented high proteolytic activity, also showed a high percentage of autolysis already after 4 h, reaching to 71.11 % and 97.67 % after 24 h, respectively (Tables 1 and 2). However, in general all bacteriocinogenic strains presented high autolytic activities, indicating that they may be good candidates for adding in dairy products. It is interesting to note that *L. lactic* subsp. *lactis* GLc05, that present a high antimicrobial activity *(28)* presented low autolytic activity (16.4% after 24 h, Table 1), which in this case would be preferable, since this strain will be able to survive longer in the product and continue to produce bacteriocins. Autolysis can be caused by autolysins or prophage endolysins: the rate of starter autolysis is an important factor controlling cheese ripening and flavor development, once many starter enzymes that affect cheese ripening are located intracellularly, such as peptidases, lipases and enzymes that catalyze amino acid conversions *(32, 33)*. Although starter autolysis is usually beneficial, undesirable consequences such as insufficient acid production and removal of residual lactose can result if autolysis is too rapid. In practice, a balance in starter autolysis is necessary for optimal cheese ripening and flavor development in some type of cheeses *(8, 32)*.

None strain was able to produce EPS (Tables 1 and 2). Some LAB strains are able to secrete EPS, extracellular polysaccharides that are economically important for the production of yogurts because they improve the rheological characteristics of the product, creating smooth and creamy texture and can confer beneficial health effects *(13)*.

L. lactis subsp. *lactis* GLc22 and 25 *Enterococcus* strains were able to produce diacetyl. The production of diacetyl demonstrated to be strain-dependent (Tables 1 and 2), because not all LAB have the capacity to metabolize citrate, so this behavior might differ between species and strains. Diacetyl is a volatile compound generated as an end product of the conversion of citrate in pyruvate, which contributes to buttery and "buttermilk" aroma and flavor in dairy products *(20, 36)*. This compound is commonly produced by some LAB strains, such as *L. lactis* var. *diacetylactis*, and also by some *Enterococcus* strains *(2, 13)*.

The ability of adaptation and survival of starter strains in different and high salt concentrations is very important during cheese production. The tolerance to different concentrations of NaCl (4, 6 and 10%) presented by lactococci and enterococci strains individually are show in Tables 1 and 2, respectively. The growth in MRS without NaCl and in MRS with NaCl 4% (w/v) was not significantly different ($p < 0.05$). However, adding NaCl at 6 and 10% (w/v) resulted in a lower growth when compared to control (MRS without NaCl) ($p < 0.05$). *L. lactis* subsp. *lactis* GLc05 and 23 *Enterococcus* strains presented a high tolerance to NaCl at 10% (w/v) (Tables 1 and 2). This finding is interesting, since these strains were isolated from raw milk, with very low salt content. Piraino *et al. (33)* has described that salt tolerance of some *Lactococcus* isolates from cheese may also reflect an adaptation to the cheese environment (2 to 10% salt-in-moisture for different Caciocavallo varieties), and that strains isolated from cheeses with lowest salt content presented low-salt tolerance. Some *L. lactis* subsp. *lactis* tested by Dal Bello *et al. (9)* were not able to grow in NaCl at 4%.

During ripening, due to proteolytic enzymes, casein is partly converted into free amino acids. The free amino acids contribute to specific sensorial characteristics in the end product, but can also be targets for decarboxylase enzymes mainly from LAB, often as an adaptation response to low pH values, resulting in production of biogenic amines (BA). According to Collins *et al. (7),* healthy volunteers exhibited no symptoms after consumption of 25 to 50 mg of histamine with fish or non-alcoholic drinks; also, in some volunteers histamine levels ranging from 75 to 300 mg in fish or nonalcoholic beverages could provoke mostly headache and flushing. They also reported an outbreak implication the intake of cheeses containing histamine ranged between 850 and 1,870 mg/kg. So, it is relevant to characterize the potential in producing BA by LAB that can be used as starter cultures and/or biopreservatives in foods, in order to assess their safe use. Lactococci and enterococci strains used in this study and previously reported as possessing encoding genes for decarboxylases production *(29)* were submitted to HPLC quantification of BA production and the results are presented in Table 3. None strain was able to produce putrescine, histamine, cadaverine, or spermidine. Twelve strains (5 lactococci and 7 enterococci) were capable to form tyramine and four strains (1 lactococcus and 3 enterococci) were capable to form 2-phenylethylamine (Table 3). The production of BA is mainly attributed to *Enterococcus* strains *(14),* but lactococci has been previously demonstrated to produce BA. Horizontal gene transfer has been proposed one of the mechanisms by which BA-producing ability is acquired by LAB *(17, 21)*. However, the produced amounts of BA by enterococci and lactococci strains in this study can be considered low (Table3). Ladero *et al. (17)* has described that biogenic amine production is a species-level trait.

Ripening cheeses represent a good environment for production and accumulation of BA produced by starter and nonstarter LAB strains. Flasarová *et al. (12)* have demonstrated that inoculation of decarboxylases producers strains in model cheeses determined high BA concentrations,

suggesting that the strains are more adaptable to difficult conditions in ripened cheese and probably use the substrate for growth and survival in the real cheese system more effectively. Tyramine is normally metabolized by monoamine oxidase enzyme (MAO) located in the digestive tract and liver. In patients treated with antidepressants using inhibitors of MAO, tyramine is not metabolized causing food migraine and hypertensive crises *(19)*. Tyramine and histamine have great impact on human health *(5)* and they are described as main BA found in cheese produced with goat milk, while 2-phenylethylamine is usually found at low concentrations *(26)*.

Histamine is controlled in fresh and cured fisheries by the European legislation at maximum levels of 200 mg/kg and 400 mg/kg, respectively, and US Food and Drug Administration (FDA) considers the concentration of 500 mg/kg as risky in these food products *(10, 11)*. For other foodstuffs, histamine concentrations are not controlled, but its presence at 100 mg/kg is considered a hazard in fermented foods *(18, 38)*. High concentrations of BA in dairy products is usually associated to poor hygiene conditions of milk production, highlighting the need of proper control of microbial contamination and choosing non-BA producers as starter cultures *(3)*.

The technological properties, like proteolytic and acidifying activity and diacetyl production, demonstrated to be strain-dependent. Some lactococci strains had a high acidifying activity, being capable to drop the pH to 5.3 or lower after 6 h. *L. lactis* subsp. *lactis* GLc06 presented a great acidifying, proteolytic and autolytic activity. Interestingly, strain GLc05 was capable to grow at high NaCl concentrations (10 % w/v), even presenting a medium autolytic and proteolytic activity; moreover it possesses an *in situ* antimicrobial activity against coagulase-positive *Staphylococcus (28)*. Even if *Enterococcus* could carry putative virulence genes, many studies have already demonstrated their biopreservative use and safe application. In the present study some strain presented the ability to produce diacetyl and a high autolytic and extracellular

proteolytic activity and also presented resistance to high NaCl concentrations (even at 10 % w/v). The present study demonstrated that some bacteriocin-producing lactococci and enterococci present interesting technological properties that can justify their use by the dairy industry: besides their technological potential, their biopreservative characteristics can represent an input in their beneficial properties.

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Table 1. Technological characterization of bacteriocinogenic lactococci isolated from raw goat milk.

Strain	strain code	Acidifying activity			EPS production*	Diacetyl production*	Extracellular proteolytic activity (mm)	Autolysis (%)**		Growth at different [NaCl]***			
		pH in UHT goat milk											
		0 h	6 h	24 h				4 h	24 h	0	4%	6%	10%
nisin producers													
Lactococcus lactis	GLc03	6.77	5.73	4.20	-	-	1	51.94	52.50	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc04	6.77	5.40	4.16	-	-	3	44.09	46.26	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc05	6.77	5.09	4.18	-	-	3	16.43	26.36	++	++	++	++
Lactococcus lactis subsp. *lactis*	GLc08	6.77	5.30	4.17	-	-	3	35.11	41.66	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc14	6.77	5.04	4.17	-	-	2	35.32	37.09	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc18	6.77	5.64	4.18	-	-	2	52.18	56.55	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc19	6.77	5.00	4.19	-	-	3	50.80	51.06	++	+	+	+
Lactococcus lactis subsp. *lactis*	GLc20	6.77	5.64	4.12	-	-	2	58.17	56.60	+++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc21	6.77	6.27	4.10	-	-	-	53.54	54.39	++	++	++	+
not identified bacteriocin													
Lactococcus lactis	GLc01	6.77	5.09	4.17	-	-	3	41.49	42.17	++	++	++	+
Lactococcus lactis	GLc02	6.77	5.47	4.18	-	-	1	41.90	42.95	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc06	6.77	5.02	4.15	-	-	4	68.88	71.11	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc07	6.77	5.71	4.19	-	-	3	39.42	43.91	++	++	+	+
Lactococcus lactis subsp. *lactis*	GLc09	6.77	5.16	4.15	-	-	3	32.86	39.83	++	++	+	+
Lactococcus lactis subsp. *lactis*	GLc10	6.77	4.90	4.16	-	-	3	29.65	36.33	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc11	6.77	4.92	4.17	-	-	2	71.11	73.33	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc12	6.77	5.65	4.17	-	-	2	17.25	24.60	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc13	6.77	4.95	4.15	-	-	3	33.54	38.33	++	++	+	+
Lactococcus lactis subsp. *lactis*	GLc15	6.77	5.12	4.14	-	-	2	35.40	47.81	++	+	+	+
Lactococcus lactis subsp. *lactis*	GLc16	6.77	5.04	4.16	-	-	3	37.23	38.43	++	++	+	+
Lactococcus lactis subsp. *lactis*	GLc17	6.77	4.87	4.16	-	-	3	39.20	50.93	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc22	6.77	5.30	4.29	-	+	3	13.12	37.06	++	++	++	+
Lactococcus lactis subsp. *lactis*	GLc23	6.77	4.99	4.16	-	-	3	47.17	51.53	++	++	+	+

*Positive result (+), negative result (-). **Percentage of autolysis was determined as: 100–(A1/A2 9100), where A1 is equal to the lowest and A2 the highest OD measured during incubation. ***Growth in NaCl measured by the spectrophotometric method- growth measurement was performed subtracting the OD value at t=0h from OD value at t=24h: ($OD_{650} \leq 0.1$ = (+); $0.1 < OD_{650} \leq 0.5$ = (++); $OD_{650} > 0.5$ = (+++).

Table 2. Technological characterization of bacteriocinogenic enterococci isolated from raw goat milk.

Strain	strain code	Acidifying activity			EPS production*	Diacetyl production*	Extracellular proteolytic activity (mm)	Autolysis (%)**		Growth at different [NaCl] ***			
		pH in UHT goat milk											
		0 h	6 h	24 h				4 h	24 h	0	4%	6%	10%
enterocin producers													
Enterococcus durans	GEn01	6.77	5.72	4.38	-	+	2	44.51	58.30	++	++	+	+
Enterococcus durans	GEn02	6.77	5.79	4.44	-	+	1	58.67	60.05	++	++	++	++
Enterococcus durans	GEn03	6.77	6.10	4.60	-	+	1	49.57	54.10	++	++	++	++
Enterococcus durans	GEn04	6.77	5.81	4.42	-	+	2	53.24	56.48	++	++	++	++
Enterococcus durans	GEn05	6.77	5.78	4.43	-	+	2	57.68	58.30	++	++	++	+
Enterococcus durans	GEn06	6.77	5.77	4.41	-	+	2	48.29	51.70	++	++	++	++
Enterococcus durans	GEn07	6.77	5.80	4.36	-	+	2	49.69	60.06	++	++	++	+
Enterococcus durans	GEn08	6.77	5.73	4.45	-	+	1	40.41	41.78	++	++	++	++
Enterococcus durans	GEn09	6.77	5.70	4.44	-	+	1	50.56	53.93	++	++	++	++
Enterococcus durans	GEn10	6.77	5.70	4.44	-	+	2	62.38	63.88	++	++	++	++
Enterococcus durans	GEn11	6.77	5.80	4.44	-	+	2	46.57	51.46	++	++	++	++
Enterococcus durans	GEn12	6.77	5.72	4.43	-	+	2	45.62	49.06	++	++	++	++
Enterococcus durans	GEn13	6.77	5.73	4.44	-	+	2	49.35	51.92	++	++	++	++
Enterococcus durans	GEn14	6.77	5.75	4.43	-	+	2	34.66	39.44	++	++	++	++
Enterococcus durans	GEn15	6.77	6.15	4.55	-	+	2	50.93	58.12	++	++	++	+
Enterococcus durans	GEn16	6.77	5.79	4.44	-	+	2	56.02	59.93	++	++	++	++
Enterococcus durans	GEn17	6.77	5.75	4.41	-	+	2	47.61	51.02	++	++	++	++
Enterococcus faecalis	GEn18	6.77	5.45	4.38	-	+	2	36.13	64.28	++	++	++	+
Enterococcus faecalis	GEn19	6.77	5.87	4.50	-	-	-	36.55	37.93	++	++	++	+
Enterococcus faecalis	GEn20	6.77	5.42	4.37	-	+	5	40.40	47.56	++	++	++	++
Enterococcus faecalis	GEn21	6.77	5.48	4.35	-	+	3	52.92	64.12	++	++	++	+
Enterococcus faecalis	GEn22	6.77	6.04	4.69	-	+	5	72.67	97.67	++	++	++	++
Enterococcus faecalis	GEn23	6.77	5.46	4.40	-	+	5	42.30	53.84	++	++	++	+
Enterococcus faecalis	GEn24	6.77	5.57	4.37	-	+	1	30.14	32.26	++	++	++	++
Enterococcus faecalis	GEn25	6.77	5.94	4.24	-	-	1	36.53	47.97	++	++	++	++
Enterococcus faecium	GEn26	6.77	6.55	5.68	-	-	-	35.60	36.22	+++	++	++	++

Enterococcus faecium	GEn27	6.77	5.68	4.62	-	-	2	38.71	39.63	+++	+++	+++	++
Enterococcus faecium	GEn28	6.77	5.57	4.47	-	+	2	48.20	48.48	++	++	++	++
Enterococcus faecium	GEn29	6.77	6.56	5.70	-	-	-	37.01	38.95	++	++	++	++
Enterococcus faecium	GEn30	6.77	6.57	5.06	-	-	-	19.57	27.23	++	+++	++	++
Enterococcus faecium	GEn31	6.77	6.58	5.86	-	+	-	47.72	53.53	++	++	++	+
Enterococcus faecium	GEn32	6.77	6.53	4.97	-	-	-	28.60	32.21	++	++	++	+
Enterococcus hirae	GEn33	6.77	6.04	4.24	-	-	3	50.53	70.16	++	++	++	++

*Positive result (+), negative result (-).

**Percentage of autolysis was determined as: 100–(A1/A2 9100), where A1 is equal to the lowest and A2 the highest OD measured during incubation.

***Growth in NaCl measured by the spectrophotometric method- growth measurement was performed subtracting the OD value at t=0h from OD value at t=24h: ($OD_{650} \leq 0.1$ = (+); $0.1 < OD_{650} \leq 0.5$ = (++); $OD_{650} > 0.5$ = (+++).

Table 3. Biogenic amines quantificafication for bacteriocinogenic lactococci and enterococci isolated from raw goat milk.

Strain	strain code	HPLC mg/kg*	
		2-phenylethylamine	tyramine
Lactococcus lactis	GLc03	0.41 ± 0.4	2.44 ± 2.15
Lactococcus lactis subsp. *lactis*	GLc05	ND	1.19 ± 2.06
Lactococcus lactis subsp. *lactis*	GLc17	ND	0.73 ± 0.09
Lactococcus lactis subsp. *lactis*	GLc20	ND	2.71 ± 1.23
Lactococcus lactis subsp. *lactis*	GLc22	ND	0.91 ± 0.01
Lactococcus lactis subsp. *lactis*	GLc23	ND	ND
Enterococcus durans	GEn03	1.85 ± 2.32	1.15 ± 1.2
Enterococcus durans	GEn09	ND	5.26 ± 0.09
Enterococcus durans	GEn16	2.59 ± 2.53	4.30 ± 0.95
Enterococcus faecalis	GEn18	ND	0.84 ± 0.02
Enterococcus faecalis	GEn22	4.92 ± 0.21	3.83 ± 0.11
Enterococcus faecium	GEn26	ND	ND
Enterococcus faecium	GEn27	ND	3.34 ± 0.38
Enterococcus hirae	GEn33	ND	1.63 ± 0.88

*putrescine, histamine, cadaverine, and spermidine: not detected