Technological characterization of bacteriocin producing strains isolated from a traditional cheese

Susana Ribeiro[*], *Márcia C. Costa*, *Airidas Dapkevicius* , *Maria de Lurdes Enes Dapkevicius* , *Célia C.G. Silva*

CITA-A, Universidade dos Acores, Angra do Heroiìsmo, Acores, Portugal
* susanaribeiro@uac.pt

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RESUMO

Technologically relevant properties of eight isolates of lactic acid bacteria (LAB) from Pico cheese, producing anti-listeria bacteriocins, were evaluated in order to consider their use as starter or adjunct cultures. The isolates were identified as *Enterococcus faecalis*, *Lactobacillus paracasei* and *Lactococcus lactis* (API50 CH), and were screened for their acidifying capacity in different media (MRS broth, Skim milk and UHT milk), enzymatic activity (API-ZYM), proteolytic and lipolytic activities, exopolysaccharide and diacetyl formation, and autolytic activity. All the strains revealed good acidifying capacity especially in Skim milk and UHT milk. Only one isolate showed proteolytic activity and two showed lipolytic activity. None of the isolates was found to produce exopolysaccharides, but most of them were able to produce diacetyl from citrate. *Lactobacillus paracasei* isolates exhibited good autolytic ability. Ability to grow at different temperatures and NaCl concentrations was also tested. Maximal growth was observed at 30^{o}C and minimum at 4^{o}C. All the isolates were able to growth in the presence of 2% and 6% of NaCl. The incidence of virulence factors as β-hemolytic, gelatinase and DNase activity and histamine production was also investigated. Only four strains showed positive gelatinase activity. Coexistence of the isolates was evaluated by a cross-streak method. Most of the isolates could coexist, although one isolate inhibited the growth of all the others isolates. Some isolates presented important technological properties for practical application as adjunct and protective cultures in cheese production.

1. INTRODUCTION

Lactic acid bacteria (LAB) are the dominant starter cultures employed in the production of fermented foods due to their contribution to flavour, texture, nutritional value and microbial safety [1]. The specific characteristics of taste and aroma of traditionally made cheese varieties may be attributed to the type of milk, its microbiological quality, the degree of acidification of the curds, the manufacturing technology and the ripening conditions. The ability to produce acid rapidly is probably the most important property of starter bacteria. However, LAB plays also a major role in releasing specific compounds responsible for cheese

flavour development. LAB contribute to proteolysis and lypolysis events in cheeses as they can degrade the products derived from rennet action on casein and hydrolyse milk fat or, at least, some triglycerides [2]. LAB autolysis in cheese is also of particular importance because it allows key intracellular enzymes involved in cheese ripening to reach their substrates more easily [2]. LAB also produces an array of antimicrobial substances such as organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins. The use of bacteriocins in the food industry can help to reduce the addition of chemical preservatives [3].

Targeted screening of natural communities may diversify the market of traditional foods and help to select LAB strains with technologically relevant properties, which will be well adapted to the microenvironment of the new product. The evaluation of these characteristics is useful to optimise cheese production made with pasteurized milk to be brought closer to those of traditional varieties and producing tastier, safer and healthier products. Therefore, the aim of this study was to evaluate the technological properties of LAB isolated from a traditional cheese (Pico cheese) in order to evaluate their potential application as starter and/or adjunct cultures for the manufacture of cheese.

2. MATERIALS AND METHODS

2.1 Microorganisms

The isolates tested were selected from LAB isolated from Pico cheese. They were chosen on the basis of their antimicrobial activity against a reference strain *Listeria monocytogenes* ATCC 7466. They were identified by the API50 CH system, as *Enterococcus faecalis* (4), *Lactobacillus paracasei* (3) and *Lactococcus lactis* (1).

2.2 Technological characterization

Acidification activity was measured by the change in pH during time and evaluated in different media, MRS broth, UHT milk and Skim milk [4]. Growth of the LAB isolates on different NaCl concentrations (2, 6 and 10%) and temperatures (4 to 45ºC) was evaluated in MRS by measuring the optical density (OD) at 630nm. Determination of exopolysaccharide (EPS) production was performed on MRS agar plates containing one of the following sugars as carbon source: glucose, fructose, sucrose and lactose [5]. Diacetyl production from citrate was determined according to King [6]. Autolysis of whole cells was determined in buffer solution (potassium phosphate, 50 mmol/L, pH 6.5) following the method of Mora *et al.* [7]. Detection of proteolytic and lipolytic activities of the isolates was made on Skim Milk Agar and tributyrin agar, respectively [4,8]. Haemolytic activity was determined by streaking the strains on plates with blood agar [9]. Gelatinase production was tested using agar plates containing gelatin [10]. The DNase Test Agar was used for detection of DNase [11]. A differential medium was used for histamine detection [12]. Coexistence among the isolates was examined by a cross-streak method [13]. The API-ZYM system (bioMérieux, France) was used to determine the enzyme profile of the eight LAB isolates.

3 RESULTS AND DISCUSSION

All the eight isolates tested presented high acidification rate after 48h, especially in milk (UHT and skim milk, data not show). In table 1 presents some technologically relevant properties of LAB isolates. LAB tested in this study showed maximum growth at 30ºC and minimum growth at 4ºC (table 1). Two of the isolates (*Lb. paracasei* and *L. lactis)* exhibit identical growth at 15ºC and 30ºC. Sodium chloride tolerance tests revealed that all isolates were able to grow at the lowest salt concentrations used (0 and 2%). *L. lactis* were not able to grow in the presence of salt concentrations above 6% NaCl (table1). LAB isolate identified as *L. lactis* was found to have greater extents of autolysis (table 1). On the contrary, the isolate with the lowest autolytic aptitude was the one identified as *Lb. paracasei* (L3A21M8). This isolate did not produce diacetyl, but was the only one to show positive proteolytic activity (table 1). In opposition, the other three *Lb. paracasei* isolates were the ones that registered the highest levels of diacetyl production (table1). Diacetyl is a flavour compound generated as an end product of citrate metabolism by certain LAB. Diacetyl production in combination with proteolytic and lipolytic activities are important for flavour development in cheese maturation. Most of the isolates showed no lipolysis activity with the exception of two isolates identified as *E. faecalis* and *Lb paracasei* (table 1).

Table 1 - Technological aspects of LAB isolates. Isolates were identified as follows: 1-L2B21K3 (*E. faecalis*), 2-L3B1K3 (*E. faecalis*), 3-L3A21K6 (*E. faecalis*), 4-L3A21K7 (*E. faecalis*), 5- L3A21M1 (*Lb. paracasei*), 6-L3A21M3 (*Lb. paracasei*), 7-L3A21M8 (*Lb. paracasei*) 8-L3A1M6 (*Lc. lactis*).

		LAB Isolates*							
		1	2	3	4	5	6	7	8
	Temp (ºC)								
Growth[a]	4	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
	15	+	+	+	+	+	+	+	++
	30	++	++	++	++	+	++	++	++
	45	+	+	+	+	+/-	+	+	+
	NaCl (%)								
Growth[a]	0	++	++	++	++	++	+	+/-	++
	2	++	++	++	++	++	+/-	+/-	++
	6	+	+	+	+	+/-	+/-	+/-	+
	10	+	+	+	+	+/-	+/-	+/-	-
	Time (h)								
Autolysis[b] (%)	4	43.1	47.8	38.9	49.9	41.9	50.1	22.6	49.9
	6	48.6	51.0	47.8	52.1	45.1	50.5	25.6	59.9
	12	52.2	51.0	56.2	54.1	46.1	50.5	31.6	63.2
	24	54.3	51.8	59.7	56.2	47.9	50.5	33.9	63.2
	48	55.0	51.8	60.3	56.2	47.9	50.5	37.4	63.2
Diacety generation[c]		+	+	++	+++	++	++	-	++
Proteolysis[d]		-	-	-	-	-	-	+	-
Lypolysis[d]		-	+	-	-	+	-	-	-

a- High growth (++), medium (+), low (+/-), negative (-)
b- Autolysis of LAB isolates is expressed in percentage (%)
c- Production of diacetyl high (+++), medium (++), low (+) and negative (-).
d- Positive result (+), negative result (-).

The application of the API-ZYM system (results not shown) revealed that most of the isolates presented a weak activity of protease trypsin and average production of α-chymotrypsin.

Enzyme leucine arylamidase was produced among all isolates in significant amounts. On the contrary, activity of the other two peptidases (valine arylamidase and cystine arylamidase) was practically nonexisting. All the isolates showed weak oxidase activity. Seven of the isolates were able to produce esterase-lipase and acid phosphatase enzymes. Alkaline phosphatase was produced in low levels.

The *E. faecalis* isolates did not showed any of the virulence factors tested. Some *Lb. paracasei* were positive for gelatinase production but negative for the other factors tested.

One *Lb. paracasei* isolate inhibit the growth of the other isolates in the cross-streak method. The *L. lactis* isolate inhibit the growth of all *E. faecalis* under study (results not shown).

4 CONCLUSIONS

In this study we characterize relevant technologically properties of eight LAB isolates exerting antimicrobial activity against *Listeria monocytogenes*. The LAB isolates could be used as an adjunct to complement the activities present in the starter, influence flavour development during cheese ripening and improve safety of the final product. They present fast acid production, autolytic activity and, in some cases, proteolytic and lipolytic activity. Further studies should be carried out to evaluate the effectiveness of selected microorganisms in cheese model systems.

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References

[1] L Setanni, A Corsetti, Int J Food Micro, 2008, 121, 123-138

[2] L González, N Sacristán, R Arenas, JM Fresno, ME Tornadijo, Food Microb, 2010, 27, 592-597

[3] P Piraino, T Zotta, A Ricciardi, PLH McSweenwy, E Parente, Int Dairy J, 2008, 81, 81-92

[4] E Franciosi, L Settanni, A Cavazza, E Poznanski,Int Dairy J, 2009, 19, 3-11.

[5] T Smitinont, C Tansakul, S Tanasupawat, S Keeratipibul, L Navarini, M Bosco, P Cescutti, Int Food Microb J, 2007, 51, 105-111.

[6] N.King, Dairy Industries, 1948, 13, 860-866.

[7] D Mora, F Musacchio, MG Fortina, L Senini, PL Manachini, Appl Microb J, 2003, 94, 561-570.

[8] E Hantsis-Zacharov, M Halpern, Appl Environ Microb, 2007, 73, 7162–7168.

[9] IA Asteri, N Robertson, DM Kagkli, P Andrewes, G Nychas, T Coolbear, R Holland, V Crow, E Tsakalidou, Int Dairy J, 2009, 19, 595–604

[10] A Terzic-Vidojevic, K Veljovic, M Tolinacki, M Nikolic, M Ostojic, L Topisirovic, Genetika, 2009, 41, 117-136.

[11] H Gupta, RK Malik, , 2007, Lait, 2007, 87, 587–601.

[12] HMLJ Joosten, MD Northold, Appl Environ Microb, 1989, 55, 2356-2359.

[13] H X Guo, JM Kim, MH Nam, SY Park, JM Kim, 2010, Anaerobe,16, 321-326.