

DETECTION AND CHARACTERIZATION OF BACTERIOCIN-PRODUCING *LACTOCOCCUS LACTIS* STRAINS

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ABSTRACT

One hundred sixty seven strains of *Lactococcus lactis* were screened for bacteriocin production by well diffusion assay of GM17 agar. Fourteen (8.4%) produced antimicrobial activity other than organic acids, bacteriophages or hydrogen peroxide. The frequency of bacteriocin production ranged from 2% in *L. lactis* subsp. *cremoris* up to 12% in *L. lactis* subsp. *lactis*. Antimicrobial activities were not observed in any strain of *L. lactis* subsp. *lactis* var. *diacetylactis*. Among thirteen bacteriocin-producing strains and two nisin-producing strains (*L. lactis* subsp. *lactis* ATCC 11454 and *L. lactis* subsp. *lactis* CNRZ 150), eight (53%) were characterized as lactose-positive (Lac⁺) and proteinase-negative (Prt⁻). The bacteriocin-producing cultures were also characterized on the basis of plasmid content. All strains had 2 to 7 plasmids with molecular weights varying from 0.5 to 28.1 Mdal. Four strains (ITAL 435, ITAL 436, ITAL 437 and ITAL 438) showed identical profiles and the other were quite distinct.

Key words: *L. lactis*, bacteriocins, fermentation, plasmids

INTRODUCTION

L. lactis has been traditionally used as starter in the manufacture of cheese and fermented milk products on account of their function of preservation and contribution to flavor and aroma. Selected strains are used as combined cultures, single or as mixture of single cultures. Preservation of fermented foods is due primarily to the conversion of sugars in organic acids with a concomitant lowering of the pH and removal of large amounts of carbohydrates as nutrient sources. These effects extend the shelf life and safety of the final product (3, 7, 26).

In recent decades, it has become clear that the overall inhibitory action is due to more complex antagonistic systems produced by the starter cultures. Those systems included the production of the hydrogen peroxide, diacetyl, secondary reaction products, and bacteriocins (3). Competition for essential nutrients, the accumulation of D-aminoacids, a lowering of oxidation-reduction potential and coaggregation may also be involved in antagonism (26).

Bacteriocins produced by lactic acid bacteria can be defined as biologically active proteins or protein complexes displaying a bactericidal mode of action

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exclusively towards Gram-positive bacteria and particularly closely related species. They form a heterogeneous group with respect to producing bacterial species, molecular size, antibacterial spectrum, mode of action, stability and physical and chemical properties (6,7).

Bacteriocin production was detected in all genera of lactic acid bacteria and have been well described for the subspecies of *L. lactis*. Kozak *et al.* (15) observed 88% of bacteriocin-producing among 67 nisin-non-producing *L. lactis* subsp. *lactis* strains. In a study with 150 *L. lactis* subsp. *cremoris*, Davey and Richardson (5) found 11 (7%) of strains diplococci-producing. Geis *et al.* (9) showed the production of bacteriocin in 65 (23.2%) of all 280 strains examined. The frequency was of 1% in *L. lactis* subsp. *lactis* var. *diacetylactis* and 9% and 7.5% in *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*, respectively. Out of 600 strains isolates from raw buffalo milk by Gupta and Batish (11), 34 (5.7%) inhibited at least one or more indicator strains. The frequency of production ranged from 3.8% in *L. lactis* subsp. *cremoris* to 6.8% and 7.6% in *L. lactis* subsp. *lactis* var. *diacetylactis* and *L. lactis* subsp. *lactis*, respectively. Piard *et al.* (24) found 18% of *L. lactis* bacteriocin-producing of all 50 strains examined. Vanderberg *et al.* (32) reported the production of nisin-like for 8 (14.5%) of all 55 strains examined. Villani *et al.* (33) founded activity bacteriocin-like in 12 (16.7%) of *L. lactis* subsp. *lactis* in a total of 72 strains.

With the emergence of psychrotrophic food-borne microorganisms, the development of new food technologies and the search of consumers for natural food products, the bacteriocins and/or producing microorganisms have been recognized as a potential source of biopreservatives for foods. The objective of this study was to evaluate the production of bacteriocins among 167 strains of *L. lactis* belonging to the Lactic Acid Bacteria Collection of the Instituto de Tecnologia de Alimentos, Campinas, SP, Brazil. Bacteriocin-producing strains were evaluated for fermentation characteristics and plasmid profiles.

MATERIALS AND METHODS

Microorganisms and culture media

The strains of *L. lactis* coming from different sources and tested for antagonistic activity are listed in Table 1. The stock cultures were maintained in frozen storage at -20°C in 14% solids and sterile

reconstituted nonfat skim milk powder. Prior to use, the cultures were transferred twice in M17 broth (Oxoid) supplemented with 0.5% glucose (GM17) and incubated at 30°C for 16-18 h. *L. lactis* subsp. *cremoris* ITAL 23, ITAL 257 and ITAL 309 were used as indicator cultures. These cultures were previously selected as the most sensitive representatives in the collection (23). The nisin-producing *L. lactis* subsp. *lactis* ATCC 11454 and *L. lactis* subsp. *lactis* CNRZ 150 were used as positive controls.

Table 1. Origin of lactic acid bacteria strains.

Number of strains	Species	Origin
1	<i>L. lactis</i> subsp. <i>lactis</i> 11454	ATCC
1	<i>L. lactis</i> subsp. <i>lactis</i> 150	CNRZ
38	<i>L. lactis</i> subsp. <i>lactis</i>	Raw buffalo milk
10	<i>L. lactis</i> subsp. <i>lactis</i>	Cheese whey
10	<i>L. lactis</i> subsp. <i>lactis</i>	Commercial cultures
12	<i>L. lactis</i> subsp. <i>lactis</i>	Cheese starters cultures
30	<i>L. lactis</i> subsp. <i>lactis</i>	Regional cheese
8	<i>L. lactis</i> subsp. <i>lactis</i>	INRA
2	<i>L. lactis</i> subsp. <i>cremoris</i>	Raw milk
8	<i>L. lactis</i> subsp. <i>cremoris</i>	Commercial cultures
1	<i>L. lactis</i> subsp. <i>cremoris</i>	INRA
38	<i>L. lactis</i> subsp. <i>cremoris</i>	Cheese starter cultures
10	<i>L. lactis</i> subsp. <i>lactis</i> var. <i>diacetylactis</i>	Commercial cultures

ATCC: American Type Culture Collection, Baltimore, MD, USA; CNRZ and INRA: Centre de Recherches Agronomiques de Jouy-en-Josas, France.

Detection of antagonistic activity

The well diffusion direct assay described by Tagg and McGiven (29) and modified by Benkerroum *et al.* (2) was utilized to detect the bacteriocin-producing strains. The GM17 broth was supplemented with sodium β -glycerophosphate (2%) and catalase (Sigma Chemical Co., Dorset, England) at final concentration of 100U. Twenty millilitres of GM17 agar inoculated with 1% (10^5 - 10^6 cfu ml⁻¹) of stationary-phase sensitive indicator cultures were poured in a sterile Petri dish and allowed to harden. Holes were punched out of the agar, by using a cork bore (4 mm of diameter). The base of each hole was sealed with a drop (50 μ l) of GM17 soft agar (0.75% agar) and then filled with 50 μ l overnight test strains. The inoculated plates were incubated at 30°C for 18-24 h and checked for the presence of inhibition zones as a result of antimicrobial activity. In addition, two other techniques for inoculation were tested. The test

producer cultures were inoculated as a single streak and as a spot onto the surface of GM17 agar, previously inoculated with the sensitive indicator cultures. The plates were incubated at 30°C for 18-24 h and were checked for inhibition zones around the spot and/or the streak.

Detection of lisogenic strains

To detect the presence of lisogenic strains the reverse side technique was used. GM17 agar plates were streaked with test producer cultures and incubated at 30°C for 24 h. The agar was detached from edges of the Petri dish with a sterile spatula and flipped on the lid. The back surface was flooded with 4.5ml of GM17 soft agar (0.75% agar) containing 500µl of a 1:10 v/v dilution of the stationary-phase indicator cultures (*L. lactis* subsp. *cremoris* ITAL 23, ITAL 257 and ITAL 309). The plates were incubated at 30°C for 24-48 h and examined for inhibition zones (30).

Fermentation characteristics of bacteriocin-producing strains

Bacteriocin-producing strains were evaluated for the ability of lactose fermentation and proteinase activity. The strains were inoculated (1%) in 11% solids and sterile reconstituted nonfat skim milk powder (RSM 11%), milk RSM 11% supplemented with milk protein hydrolysate (0.25%), milk RSM 11% supplemented with glucose (1%) and milk RSM 11% supplemented with both. The series were incubated at 21°C for 16-48 h and examined for the presence of coagulation and pH (21, 25).

Plasmid profiles of the bacteriocin-producing strains

Plasmid profile was obtained in order to observe homology between the bacteriocin-producing strains. Cells were grown in GM17 broth at 30°C for 6 h. Plasmid extraction and agarose gel electrophoresis followed the method of Anderson and McKay (1) and Maniatis *et al.* (19). Plasmid profile of *L. lactis* subsp. *lactis* ATCC 11454, previously reported (10), was used as a standard.

RESULTS AND DISCUSSION

Screening for antibacterial activity

The assays for the presence of bacteriocins were designed to minimize the effect of other interfering

inhibitors. For example, to exclude the possibility of an antagonistic effect on indicator strain due to hydrogen peroxide, catalase was added to GM17 agar (4, 11, 24, 30). Likewise, to exclude the inhibition by low pH due to the production of organic acids, the solid media was supplemented with sodium β-glycerophosphate (5, 14, 27, 30). Among 167 strains of *L. lactis* screened for antibacterial activity against three indicator strains (*L. lactis* subsp. *cremoris* ITAL 23, ITAL 257 e ITAL 309), fourteen (8.4%) inhibited the growth of all indicator cultures. The average of the inhibition zone diameters for each strain tested is shown in Table 2.

Table 2. Inhibitory activity of 14 strains of *L. lactis* against selected indicator strains

Microorganisms	Indicator cultures		
	ITAL 23	ITAL 257	ITAL 309
<i>L. lactis</i> subsp. <i>cremoris</i> ITAL 402	++	++	+
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 104	+++	++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 179	+++	+++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 185	+++	++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 187	+++	+++	+
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 383	++	++	+
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 387	+++	++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 403	+++	++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 404	+++	++	+
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 408	++	++	+
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 435	+++	+++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 436	+++	+++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 437	+++	+++	++
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 438	+++	+++	++

+: Weak inhibition (zone of 4mm of diameter); ++ Moderate inhibition (zone of 5-9mm); +++: High inhibition (zone of 10-15mm)

The comparison among the methods of simultaneous antagonism assay (inoculation in spot, streak and wells) showed that the inoculation in wells lead to the formation of larger and more consistent inhibition zones. Indeed, these methods allowed the standardization of the inoculum, making possible the comparison between diameters of the inhibition zones (29). Benkerroum *et al.* (2) had concluded that this method is suitable and designated "lyse zones" as opposed to the spot-on-the-law method. The lyse zones could be seen after 3 h of incubation by wells diffusion method, while 48 h were necessary to see definitive inhibition zones by the spot test assay or streak inoculation. However, Lewis *et al* (17) reported that the spot

method was more reproducible, rapid and easy to score than flip-plate or well diffusion methods. Spelhaug and Harlander (27) also found the spot method superior to flip-plate method assay in a study of three bacteriocin-producing strains against a large panel of food-borne microorganisms. The antagonism deferred method for inoculation in points was more suitable when compared with the streak and wells methods due to its reproducibility, rapidity and simplicity (17).

Detection of lisogenic strains

Lisogenic strains and bacteriocin-producing strains show similar spots. The differentiation between them is difficult, which interferes with the result evaluation (20, 30, 34). An important differential characteristic is that bacteriocins, unlike bacteriophages, do not carry the genetic determinants necessary for self-replication within susceptible organisms. Only the bacteriophages can propagate in sensitive strains (20, 22, 30).

Based on the capacity of bacteriocins to diffuse in agar, the reverse technique, which eliminated the contact between the producers and sensitive strains, was used for lyse differentiation (30). The formation of the diffusion zones was observed after incubation of the Petri dishes, previously streaked with the producer strains and subsequent addition of the soft agar seeded with the sensitive strains, in the reverse side. This indicated that the substance diffuses into the agar (30). Alternatively, the observation of the characteristics of the inhibition zones helped in the differentiation of the nature of the inhibitory substance. Increasing the dilution factor resulted in reduction of the diameter of the inhibition zones until its total disappearance, indicating the presence of bacteriocins. In high dilutions the formation of the lysis plaque, characteristic of the presence of bacteriophages or bacteriocin activity, was not observed, whereas if present, phages would still be detected at much higher dilution (16, 20).

Of the 167 strains tested, 14 (8.4%) showed bacteriocin activity against all to the indicator cultures (Table 3). Thirteen (92.9%) strains are *L. lactis* subsp. *lactis* and only one (7.1%) strain is *L. lactis* subsp. *cremoris*. The frequency of bacteriocin production ranged from 2% in *L. lactis* subsp. *cremoris* to 12% in *L. lactis*. None of the *L. lactis* subsp. *lactis* biovar. *diacetylactis* strains tested in the study was able to produce bacteriocins.

Table 3. Occurrence of bacteriocin-producing *L. lactis*.

Species	Number of strains		%
	Tested*	Sensitive	
<i>L. lactis</i> subsp. <i>cremoris</i>	49	1	2
<i>L. lactis</i> subsp. <i>lactis</i>	108	13	12
<i>L. lactis</i> subsp. <i>lactis</i> var. <i>diacetylactis</i>	10	0	0
Total	167	14	8.4

* By the well-diffusion assay

The screening for the detection of bacteriocin-producing strains is highly dependent on several factors: testing method, composition of the culture medium and nature of the indicator culture. The choice of the indicator (bacteriocin-sensitive strain) used in the screening is very important and may greatly influence the results (22). Therefore, the confrontation of the results achieved with those of others authors is very difficult. Nevertheless, the occurrence of bacteriocin-producing lactococci in this study is similar to that found in the literature: 8% Vandenberg *et al.* (32), 5.7% Gupta and Batish (11) and 7.3% Davey and Richardson (5). In counterpart, these results are higher than those obtained by Zezza *et al.* (35) 1.5% or Uhlman *et al.* (31) 1.6% and lower than those from Villanni *et al.* (33) 16.7%, Piard *et al.* (24) 18%, Geis *et al.* (9) 25% and Kozak *et al.* (15) 88%.

Table 4 shows the origin of the bacteriocin-producing strains of *L. lactis* selected in this study. Among 13 *L. lactis* subsp. *lactis* strains, 6 (46.1%) were isolated from raw milk, 3 (23.1%) from regional cheese and 4 (30.8%) from industrial lactic starters. *L. lactis* subsp. *cremoris* was isolated from industrial lactic starters.

Table 4. Origin of *L. lactis* bacteriocin-producing strains.

Origin	Species (%)	
	<i>L. lactis</i> subsp. <i>cremoris</i>	<i>L. lactis</i> subsp. <i>lactis</i>
Raw milk	0.0	46.1
Regional cheese	0.0	23.1
Cheese starter cultures	2.0	30.8

Fermentation characteristics of bacteriocin-producing strains

The fermentation characteristics of bacteriocin-producing strains were evaluated after 24 h at 21°C in 11% RSM milk and the same medium

supplemented with 1.0% glucose, 0.25% milk protein hydrolysate or both. The nisin-producing strains *L. lactis* subsp. *lactis* ATCC 11454 and *L. lactis* subsp. *lactis* CNRZ 150 were also included in this test. Seven cultures were classified as fast strains (ITAL 104, ITAL 179, ITAL 187, ITAL 403, ITAL 404, ITAL 408 and CNRZ 150). They coagulated milk within 16 h at 21°C. Eight strains required 16 h or more to coagulate milk, and were classified as slow (ITAL 185, ITAL 383, ITAL 387, ITAL 435, ITAL 436, ITAL 437, ITAL 438 and ATCC 11454). Slow variants may be defective in lactose metabolism (Lac⁻) and/or proteinase activity (Prt⁺). These characteristics are carried on plasmids in some strain (13).

In glucose-supplemented milk, the slow cultures remained defective in proteolytic enzyme activity but did lower the pH, indicating that glucose was being fermented. When the milk was supplied with an exogenous nitrogen source, the cultures showed slow fermentation of glucose as evidenced by the decrease in pH. To determine the characteristic Prt⁻, the cultures were grown in milk supplemented with glucose or glucose plus an exogenous nitrogen source. The cultures were Prt⁻ since that had little, if any, proteolytic activity. This was confirmed by low pH that did occur if the glucose was supplemented with a nitrogen source. This indicated that the inability to produce acid from glucose was due to the inability to acquire needed nitrogenous compounds (12, 21, 25).

Eight (53%) out of 15 strains were characterized as lactose-positive and proteinase-negative (Lac⁺Prt⁻)

(Table 5). Vandenberg *et al.* (32) showed that 21.6% of lactic acid bacteria isolated from different sources lacked any sign of extracellular proteolytic activity. This confirmed the inability of bacteriocin-producing lactococci to grow and to produce acid at rates suitable for cheese manufacture. The failure acid production by lactococci when grown in milk is due to the lack of the proteinase enzyme system (8, 18, 25). Strains lacking this enzyme system are designated as proteinase-negative, and they grow in milk only until soluble nitrogenous compounds are depleted (25). The reasons for the lack of proteinase activity among nisin-producing lactococci are the loss of the proteinase and incompatibility of the genes for proteinase activity with the genetic elements encoding bacteriocin production (25, 28).

Plasmid profiles of the lactococci bacteriocin-producing

Table 6 shows the molecular weight of the plasmids found in bacteriocin-producing strains selected in this study. The *L. lactis* subsp. *lactis* ATCC 1454 contain six plasmids with molecular weights of 31.8, 29.1, 21.9, 19.4, 3.6 and 1.49 Mdal (10). Agarose gel electrophoresis of isolated DNA samples revealed two to seven plasmids in these cultures. Their molecular weights ranged from 0.5 to 28.1 Mdal when compared with standard plasmids (Table 6). No plasmids larger than 100 Mdal were observed. The strains *L. lactis* subsp. *lactis* ITAL 435, ITAL 436, ITAL 437 and ITAL 438 showed similar plasmid profiles. These cultures were isolated

Table 5. Number and molecular weight of plasmids found in bacteriocin-producing *L. lactis* subsp. *lactis* strains.

Bacteriocin-producing strains	Number	Plasmids
		Molecular weight (Mdal)
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 104	5	26.4; 25.9; 14.2; 9.4; 0.7
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 179	4	26.8; 25.9; 9.8; 0.5
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 185	5	26.4; 23.7; 14.6; 8.9; 3.8
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 187	4	25.5; 24.6; 23.7; 9.8
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 383	2	27.7; 25.9
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 387	5	28.1; 26.8; 23.7; 13.8; 2.9
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 403	7	27.7; 26.8; 25.5; 23.7; 20.7; 9.4; 1.2
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 404	4	26.8; 25.9; 9.4; 1.2
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 408	4	28.1; 27.2; 13.8; 5.1
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 435	4	28.1; 26.8; 23.7; 22.4
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 436	4	28.1; 26.8; 23.7; 22.4
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 437	4	28.1; 26.8; 23.7; 22.4
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 438	4	28.1; 26.8; 23.7; 22.4

from raw milk and may be derived from the same strain.

Table 6. Fermentation characteristics of bacteriocin-producing lactococci at 21°C for 24 h in 11% RSM, supplemented or not with 1.0% glucose, 0.25% milk protein hydrolysate or both.

Bacteriocin-producing strains	Fermentation characteristics*
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 104	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 179	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 185	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 187	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 383	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 387	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 403	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 404	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 408	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 435	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 436	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 437	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ITAL 438	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> CNRZ 150	Lac ⁺ Prt ⁻
<i>L. lactis</i> subsp. <i>lactis</i> ATCC 11454	Lac ⁺ Prt ⁻

* Lac⁺Prt⁻: Lactose-positive and proteinase-negative

** Lac⁺Prt⁺: Lactose-positive and proteinase-positive

On the basis of plasmid profiles the bacteriocin-producing strains were classified in three categories according to Gupta and Batish (11). Group I (low plasmid group) comprised one strain (ITAL 383), with one to three plasmids. Group II (moderate plasmid group) comprised eight strains (ITAL 104, ITAL 179, ITAL 185; ITAL 187, ITAL 387, ITAL 404, ITAL 408; ITAL 435, ITAL 436, ITAL 437 and ITAL 438), with four to five plasmids. Group III (high plasmid group) comprised one strain (ITAL 403), with six or more plasmids.

Our results showed that antagonistic substances produced by the tested strains are bacteriocins and suggest that bacteriocin-producing lactococci are present in low number in the environment. These substances were heat resistant and their proteinaceous nature was demonstrated by sensibility to proteolytic enzymes (23). They also demonstrate the inability of bacteriocin-producing lactococci to grow and to produce acid at rates suitable for cheese manufacture. Due to these factors, the use of bacteriocin-producing starter have not been fully successful in dairy products

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RESUMO

Detecção e caracterização de *Lactococcus lactis* produtores de bacteriocinas

Um total de 167 linhagens de *L. lactis* foi selecionado para os testes de produção de bacteriocinas pelo método de difusão em poços em agar GM17. Desse total, 14 (8.4%) produziram substâncias inibidoras que não foram associadas com ácidos orgânicos, peróxido de hidrogênio e bacteriófagos. A frequência de produção de bacteriocinas variou de 2% em *L. lactis* subsp. *cremoris* a 12% em *L. lactis* subsp. *lactis*. Nenhuma das linhagens de *L. lactis* subsp. *lactis* var. *diacetylactis* produziu substâncias inibidoras. De 13 linhagens produtoras de bacteriocinas e duas de nisina (*L. lactis* subsp. *lactis* ATCC 11454 e *L. lactis* subsp. *lactis* CNRZ 150), 8 (53%) foram caracterizadas como lactose-positivas (Lac⁺) e proteinase-negativas (Prt⁻). As linhagens produtoras de bacteriocinas também foram caracterizadas no seu conteúdo de plasmídios. Elas apresentaram de 2 a 7 plasmídios, com pesos moleculares aproximados de 0.5 a 28.1 Mdal. Quatro linhagens (ITAL 435, ITAL 436, ITAL 437 e ITAL 438) mostraram idênticos perfis de plasmídios. Os perfis das linhagens restantes foram distintos.

Palavras-chave: *L. lactis*, bacteriocinas, fermentação, plasmídios

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