Screening of Bacteriocin-Producing Lactic Acid Bacteria Isolated from West Algeriangoat's Milk

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Abstract: Fifty ninelactic acid bacteria isolated from Algeriangoat's milk and previously selected according to their technological properties. They were screened for antimicrobial activity. Between 3258bacterial couples brought into studies,we observe 747 cases of inhibitions (22.93%). The results obtained show that the lactobacilli have high spectrum of action. Thus, for the various species: *Lc. lactis* subsp. *lactis* (40.1%); *Lc. lactis* subsp. *lactis* biovar. *diacetylactis* (29.1%); Leuconostoc (25.8%); *Streptococcus* (56.9%); *Pediococcus* (36.5%) and Lactobacillus (13.5%). For the whole of the interactions brought into studies, the lactobacilli inhibited 392 strains of 1331 couples (29.5%); the other species have a more reduced spectrum of action: Lactococcus (18.76%); *Leuconostoc* (25.7%); *Streptococcus* (17.5%) and *Pediococcus* (5.34%). Six strains showed inhibitory activity in solid medium (well diffusion assay) when tested against the effects of organic acid and hydrogen peroxide were eliminated. These strains did not show inhibitory activity after treatment with proteinase K, trypsin or α-chymotrypsin. *Lactococcus lactis* subsp. lactis LCL01 produced a heat stable substance with a proteinaceous nature and with bactericidal action, suggesting a bacteriocin-like. Only *Lb. plantarum* LPL01 inhibit *E. coli*.

INTRODUCTION

The isolation and characterization of news strains of lactic acid bacteria from various biotopes took a great interest these last decades (Bizzarro *et al*., 2000; Saidi *et al*., 2002; Wouters *et al*., 2002; El-Soda *et al*., 2003; Ayad *et al*., 2004).

Lactic acid bacteria are traditionally used as starters for food fermentations. Since, they have a capacity to inhibit spoilage and pathogenic bacteria (Pucci *et al*., 1988; Piard *et al*., 1992; Cleveland *et al*., 2001; Ghrairi *et al*., 2004; Guessas *et al*., 2005), they are important in food preservation and intestinal prophylaxis. Lactic acid bacteria are the most important groups for industrials purposes, since, their ferment active activity involves a notable preservative capacity as a result of the drop in the pH and the antimicrobial activity of metabolites such as lactic and acetic acid, diacetyl or bacteriocins. Many Lactic Acid Bacteria (LAB) produce antimicrobial peptidesknown as bacteriocins which are directed mainly to inhibit the growth of related species or species with the same nutritive requirements (Tagg *et al*., 1976; Klaenhammer, 1993; De Vuyst, 1995; Jack *et al*., 1995; Todorov and Dicks, 2005).

Some bacteriocins have been used to inhibit this pathogen in food, either through bacteriocin-producing cultures (Nes and Holo, 2000; Garneau *et al*., 2002) or by the addition of pure or semipure bacteriocin preparations (Biswas *et al*., 1991). Many Lactic Acid Bacteria (LAB) produce antimicrobial peptides known as bacteriocins which are directed mainly to in hi bit the growth of related species or species with the same nutritive requirements (Herreros *et al*., 2005). Many lactic acid bacteria, including members of the genera Lactococcus, Lactobacillus, *Carnobacterium*, *Enterococcus* and *Pediococcus*,are known to secrete small, ribosomally synthesized antimicrobial peptides called bacteriocins (Jamuna *et al*., 2005), many of theminhibit *Listeria monocytogenes* (Ghrairi *et al*., 2004; Lash *et al*., 2005). Some bacteriocins have been usedto inhibit this pathogen in food, either through bacteriocin-producin gcultures (Todorov and Dicks, 2005) or by the addition of pure or semi pure bacteriocin preparations.

The aim of this study is the search for bacteriocins produced by lactic acid bacteria isolated from Algerian goat's milk. The objectives of the present paper are follow: to determine the nature of lactic acid bacteria from raw goat's milk of West Algeria; to study the antibacterial potential of wild isolates of LAB; to characterize the main properties of this bacterial inhibitor in the crude extract and to determine the range of antimicrobial activity of LAB against a variety of others microorganisms.

MATERIALS AND METHODS

Bacterial strains: The following 59 strains of LAB tested for their antagonistic activity were isolated from the Algerian goat's milk: *Lc. lactis* subsp. lactis (eighteen strains), *Lc. lactis* subsp. lactis biovar. diacetylactis (four strains), *Ln. mesenteroides* subsp. dextranicum (five strains), *Sc. thermophilus* (five strains), *Pc. acidophilus* (three strains), Lb. plantarum (seven strains), *Lb. salivarus* (six strains), *Lb. brevis* (six strains) and *Lb. helveticus* (five strains). The procedures for isolating, identifying, technologically characterizing and selecting these strains were those described in earlier work by Saidi. LAB isolated from goat milk was cultured, respectively in MRS broth or M17 broth at 30°C. Antagonism determinations were performed on MRS or M17.

Antagonistic substances detection: Each set of master plates was replicated three times with a multi-inoculator on MRS or M17 agar and incubated at 30°C for 18 h. The replica plates were overlaid with molten agar seeded with other strain and incubated. Plates were then examined for zones of inhibition. We sought the zones of inhibition of growth which results in clear rings around the strains sown into key. All strains demonstrating antagonism were transferred from the master plates, purified and stocked in 20% glycerol at -20°C.

Research of the nature of the inhibiting agent: Inhibitions can be caused by several agents such as acidity, hydrogen peroxide, phages and bacteriocins. The research of the nature of the inhibiting agent was started in solid and liquid medium.

Acidity production: The multiplication of LAB is accompanied by a production of acid causing the reduction in the intracellular and the extracellular pH. To minimize the acid production, we used LBP medium containing 0.25% glucose and plugged with buffer phosphate.

Hydrogen peroxide production: To detect the production of H_2O_2, one carries out cultures in thepresence of catalase at a rate of 1 mg mL^{-1} of medium. The enzyme and the indicator strain are mixed in the semi-hard medium (0.8% agar). After incubation, the reading of the results is done by comparison withthe control without catalase.

Detection of lytic bacteriophage: To detect the presence of lytic bacteriophage, a portion of the clearing zone was cut from a spot deferred antagonism assay plate. The agar plug was added to 3 mL of broth and macerated with a sterile medium. The mixture was held at room temperature for 1 h. A 100 µL amount of the suspension and 100 µL of an indicator strain (grown overnight) were suspended in 8 mL of soft (0.8%) agar. The soft-agar suspended was poured evenly over an agar plate and incubated overnight at 30°C. The formation of plaques was indicatingthe phage activity.

Bacteriocins production: The effect of the proteolytic enzymes on the inhibiting activity of the selected strains was carried out at the same time on liquid medium and solid medium. To ensure itself of the protein nature of the inhibiting substances, we used the proteolytic enzymes: pronase, α-chymotrypsin and trypsin. Each enzyme is dissolved in plug phosphates buffer (10 mM, pH 7.0) with a concentration of 10 mg mL^{-1} and sterilized by filtration (0.45 µm). During the treatment by the pronase, the trypsin and the α-chymotrypsin, the filtrate containing these enzymes is incubated during 1hour with 37°C. The sensitivity of a antibacterial substance to a given enzyme is appreciated by determining the residual activity by measurement of the diameter of zone of inhibition.

Preparation of culture supernatants: Sterile cell-free culture was obtained by centrifugation (10000 g for 15 mn at 4°C) and filtration through a 0.45 μm pore-size filter (Millipore). They were adjusted to pH 7.0 with NaOH 2 mol L^{-1}, to eliminate any effect of acidity. Inhibitory activity due to hydrogen peroxide was suppressed by the addition of catalase (3600 U mL^{-1}, Sigma). Filtrates were also treated with trypsin, α-chymotrypsin and protease (Sigma Chemical Co.). Enzymes were filter-sterilized in 50 mmol L^{-1} phosphate buffer, pH 7.0. Commercial protease preparations were used at a 1 mg mL^{-1} final concentration. Sample and blanks were incubated at 37°C for 1 h and added to crude bacteriocin preparations at final concentration of 1 mg mL^{-1}. The supernatant of 500 mL of two strains was concentrated 10-fold by using a rotavapor. The concentrated culture supernatant was used as a source of bacteriocin-like substance.

Kinetics of growth: The antimicrobial effect of supernatant was tested against the indicator strains in liquid medium M17 (20 mL) added, filtrate concentrated 10 fold or not concentrated (1%) with treatment or no was inoculated with 200 μL from overnight culture of indicator strain. At interval, samples were removed for measurement of absorbance at 660 nm.

Agar well diffusion method: LAB cultures were screened for antagonistic substances detection by the agar well-diffusion method (Tagg *et al.*, 1976). Wells were cut with a sterile tube (8 mm in diameter) in agar media plates seeded with an indicator culture. The culture supernatant (50 μL) obtained previously was placed into the wells with or without treatment. After diffusion of the supernatant into the agar (4 h at 4°C), the agar plats were incubated overnight at the appropriate temperature. The assays were performed at a final concentration of 1mg mL^{-1} for all enzymes. Samples with and without were held at appropriate temperature for 1hour. The remaining activity in both samples after enzymes digestion was detected by the agar well-diffusion method, against sensitive indicator.To test for heat activity, culture supernatant was heated at 121°C for 15 min.

Mode of action: The purpose of the study achieved is to see whether the antibacterial substance produced by *Lactococcus lactis* subsp. lactis (Lc01) has indeed a bactericidal effect or abacteriostatic effect. It is enough for that to follow the evolution of the concentration in viable indicating bacteria in the culture medium M17 liquid. A stability of the concentration in viable bacteria shows abacteriostatic effect whereas a reduction in this concentration indicates a bactericidal effect (Klaenhammer, 1988). Spectrum of activity: twenty strains were screened for activity against *Enterococcus* (ten strains), *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

RESULTS

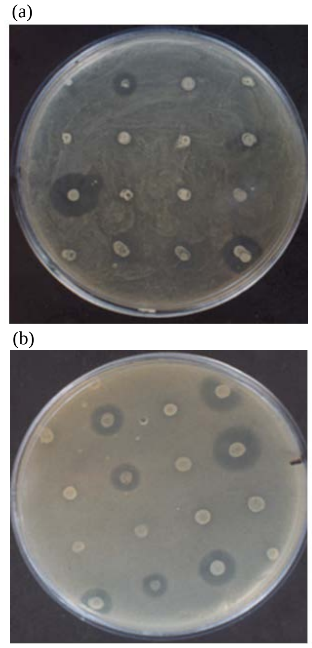
Screening for bacteriocinogenic LAB: The results consist in measuring the ray of inhibition by the indicating strains. Figure 1 show the aspect of the preparations. The whole results obtained are gathered in Table 1.

Table 1: Interaction between LAB isolated from Algerian goat milk

	Strains inhibited					
Strains inhibiting	Lc.	Ln.	Sc.	Pc.	Lb.	Total
Lactococcus						
NC	484	110	110	66	429	1199
NI	121	21	32	12	39	225
%	25	19.1	29.1	18.2	9.09	18.77
Leuconostoc						
NC	110	25	25	15	105	280
NI	40	2	5	0	25	72
%	36.4	8	20	0	23.8	25.71
Streptococcus						
NC	110	25	25	15	105	280
NI	22	5	6	0	16	49
%	20	20	24	0	15.2	17.50
Pediococcus						
NC	66	15	15	9	63	168
NI	9	0	0	0	0	9
%	13.6	0	0	0	0	5.35
Lactobacillus						
NC	514	124	123	74	496	1331
NI	196	32	70	27	67	392
%	38.1	25.8	56.9	36.5	13.5	29.45
Total						
NC	1284	299	298	179	1198	3258
NI	388	60	113	39	147	747
%	30.2	20.1	37.9	21.7	12.3	22.93

NC: Number of Couples; NI: number of inhibitions; %: percentage of inhibitions

(a)



(b)

Fig. 1(a, b): Interaction between LAB (different indicator strain: (a) *Lb. salivarus* LBS01 and (b) *Lc. lactis* subsp. lactis LCL09).

On 3258 bacterial couples brought into experiments, we observe 747 cases of inhibitions (22.93%). It is noted that *Lc. lactis* subsp. *lactis* was the most inhibiting species among the Lactococci; it is responsible for 107 inhibitions from 121 (88.43% of the cases). *Lc. lactis* subsp. lactis biovar. diacetylactis is responsible for only 14 inhibitions (11.57%). The spectrum of action of *Lc. lactis* subsp. lactis is broader than that of *Lc. lactis* subsp. lactis biovar. diacetylactis. By way of comparison, the percentages of inhibition of the various species brought into experiment: Leuconostoc (21.1%); Streptococcus (30%); Pediococcus (16.7%) and Lactobacillus (11.3%) case of *Lc. lactis* subsp. lactis, while for *Lc. lactis* subsp. lactis biovar. diacetylactis, we respectively have 10, 25, 25% and no inhibition of Lactobacillus.

We notice that the lactobacilli have high spectrum of action. Thus, for the various species: *Lc. lactis* subsp. lactis (40.1%); *Lc. lactis* subsp. lactis biovar. diacetylactis (29.1%); Leuconostoc (25.8%); Streptococcus (569%); Pediococcus (36.5%) and Lactobacillus (13.5%). For the whole of the interactions brought into experiment, the lactobacilli inhibited 392 strains from 1331 (29.5%); the other species have a more reduced spectrum of action: Lactococcus (18.76%); Leuconostoc (25.7%); Streptococcus (17.5%) and Pediococcus (5.34%). This great inhibiting effect was due to production of the lactic and/or acetic acid.

Nature of inhibitory agent: The LAB modifies the medium in such a manner that the development of other bacteria becomes impossible. This comes from the formation of lactic acid and/or acetic acid, hydrogen peroxide, phages, substances like antibiotics. In order to determine the nature of inhibitions, we were brought to check all these causes.

Acidity and hydrogen peroxide production: The acid production is responsible of six cases of inhibition (40%).We noted fourtypes of responses (Table 2): the inhibition is lostwith LCL05, LCL13, LNM04, LBP01; and LBH01; the activity was decreasedwith LCL18 and LCN04; an increase of inhibition with LCL10 and the inhibition is maintained with LCL01, LCL10, LCL18, SCT05, LBP02 and LBH03. Nine cases of inhibition were observed when the catalase is added to M17 or MRS. 46.7% of inhibition can be attributed to the hydrogen peroxide production.

Our results concord with the literature, some strains have the capacity to produce acid and/or hydrogen peroxide which inhibit functions and will stop the bacterial growth. By combining the two tests, we have observed: inhibition with only acidity or hydrogen peroxide production; inhibition with acidity and hydrogen peroxide production; some strainsinaddition to the inhibition with acidity and hydrogen peroxide production, synthesis another inhibitory substance and another factors is responsible for inhibition.

Table 2: Nature of inhibitory agent (indicator strain: *Lc. lactis* subsp. lactis LCL09)

				Medium added with 1mg mL^{-1} of			
Strains	Codes	M	MT	Ca	C	P	T
Lactococcus lactis	LCL01	5	5	5	0	0	0
subsp. lactis	LCL05	4	0	3	4	0	0
	LCL10	6	9	5	0	4	0
	LCL13	6	0	5	7	5	0
	LCL14	7	6	0	0	0	4
	LCL18	9	5	3	0	0	0
Biovar. diacetylactis	LCN04	6	3	0	5	0	4
Ln. mesenteroides subsp.	LNM01	4	5	0	4	4	5
dextranicum	LNM04	4	0	0	5	5	4
Sc. thermophilus	SCT05	5	5	6	0	0	0
Lactobacillus plantarum	LBP01	6	0	0	5	5	5
	LBP02	6	6	5	7	0	7
	LBP03	6	0	0	0	7	4
Lactobacillus helveticus	LBH01	5	0	1	5	0	0
	LBH03	6	6	7	0	0	0

M: Medium M17 or MRS no treated, MT: Medium plugged at pH7, Ca: medium added with catalase, C: medium added with α-chymotrypsin, P: medium added with protease, T: medium added with trypsin

Lytic phages production: We have detected that the inhibitory agent was the phage.

Bacteriocin-like production: Some inhibiting substances were characterized as being antimicrobial proteins. So, they were sensitive to the action of the proteolytic enzymes. For six strains (Table 2), the nature of antimicrobial substance is proteinaceous. We have different responses to the action of proteolytic enzymes.The inhibitory agent is: sensitive for the three enzymes for LCL01, LCL18, SCT05 and LBH03; sensitive only to the protease that belong toLCL10, LCN04 and LBP02 and resistant only to the protease of LCL10.

For two strainsinaddition to effect of acidtiy, the antimicrobial substance was also proteinic nature and this was resistant only to α-chymotrypsin with LCL05; sensitive only to the trypsin with LCL13.We noted that for two strains (LNM01 and LNM04), the acidity or/and hydrogen peroxyde production were the main inhibitory agents.

In liquid medium: This agent should be found in the medium where was cultivated the indicating strain. We can show it while following the curve of growth of an indicator strain in a culture medium where we added concentrated filtrate 10 times or either pure, treated in order to show the exact nature of the inhibiting agent. Only one filtrate concentrated 10 times gave us conclusive results (Fig. 2). Inhibition by *Lc. lactis* subsp lactis (LCL01) is maintained with supernatant at pH 7, added with catalase orprotease orα-chymotrypsin. The inhibition observed in solid medium added with trypsin is loss in liquid medium. It is that inliquid medium, the bacteriocinsare in a chemical configuration which makes the action of the proteolytic enzymes.

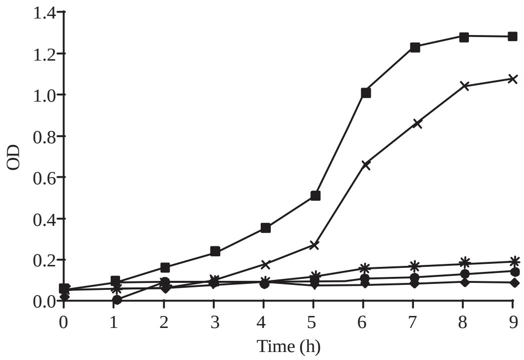


Fig. 2: Kinetics of growth of *Lc. lactis* subsp. lactis (LCL09) with the filtrate of *Lc. lactis* subsp. ■ lactis LCL01: × M17: *M17 added with filtrate at pH 7 and catalase: ○M17 added with filtrate at pH 7 and α-chymotrypsin: ◇M17 added with filtrate at pH 7 and trypsin: M17 added with filtrate at pH 7 and protease

These results indicated that the inhibitory agent was a protein and therefore,we suggested a bacteriocin-like.The inhibitory activities of culture supernatants were not modified after a treatment for 60 min at 120°C. Inhibitory activity was fully observed for supernatants which adjusted pH values 7 and with catalase. Inhibitory activity was totally lost by proteinase K, α-chymotrypsin and trypsin. This result suggests that a heat-stable proteinaceous compound was responsible for the inhibitory activity of the culture supernatant of *Lc. lactis* subsp. lactisLCL01.

Agar well diffusion method: The results obtained confirm that the inhibitory factor is, therefore, a substance of a proteinaceous nature. No inhibition zone was detected after treatment with the enzymes (Fig. 3). Since, no change was observed upon treatment with filtrate at pH 7 and filtrate added with the catalase.

Mode of action: The viability of the bacterial indicator *Lc. lactis* subsp. lactis LCL11 in the supernatant incubated at 30°C with Lc. lactissubsp. lactisLC01 is measured. After only one hour of incubation, a reduction of growthwas observed (initially the ODwas 0.18 of the concentration and. Afterbecome 0,3 after two hour of incubation (Fig. 4), this concentration decreases to 0.1. The antibacterial substance produced by LCL01 thus presents a mode of action of the bactericidal type and this bactericidal effect is relatively fast. The speed of the bactericidal effect is a characteristic of the majority of the bacteriocins. Our results concorded with those obtained by the researchers Piard *etal.* (1990) and Bhunia *et al.* (2008).

Spectrum of activity: We tested twenty strains to other species including bacteria of Gram-positive

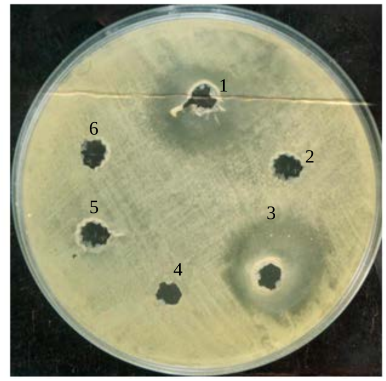


Fig. 3: Inhibition of *Lc. lactis* subsp. lactis LCL09 by LCL01 culture supernatant by the agar well diffusion assay. Wells contained cell-free supernatant was treated: well 1: at pH 7, well 2: heated for 60 min at 120°C well 3: adding catalase (1 mg mL^{-1}) well 4: adding trypsin (1 mg mL^{-1}) well 5: adding α-chymotrypsin (1 mg mL^{-1}) and well 6: adding protease (1 mg m^{-1})

Fig. 4: Spectrum of action of LAB against *E. coli*. (Only one strain inhibit *E. coli* is *Lactobacillus plantarum* LBP01)

(*Enterococcus*, Bacillus cereus and *Staphylococcus* aureus) and bacteria of Gram-negative (*E. coli*). According to Tagg *et al*. (1976), the spectrum of activity of the bacteriocins bacteria Gram-positive, although it can be variable according to thestrains, never relates to the bacteriaGram-negative. Our results showed that the bacteriocin-like do not inhibit the growth gram-negative bacteria such E.coli. Also with an aimof determining the field of activity of the antibacterial substance produced by, the sensitivity of various bacterial strains to this antibacterial substance is evaluated. On the other hand, E. coli is the only one which been affected by the inhibiting substance produced by Lb. plantarum LBP01 (Fig. 4) which has a characteristic of the bacteriocin-like. *Bacillus cereus* and *Staphylococcus aureus* were inhibited by fourteen tested strains.

DISCUSSION

The objective of the present study was to make an extensive screening program of lactic acid bacteria isolated from algerian goat's milk, in order to demonstrated antagonistic activity (Badis *et al*., 2004). The knowledge of the interactions between lactic acid bacteria remains a significant criterion for strains selection used in industrial fermentations.The research of the inhibiting capacity in the lactic acid bacteria enabled us to show that there are interactions between the various bacteria. From our study, we obtained 747 cases of inhibitions (22.93 %); this percentage is relative because it depends on the culture conditions and also on the indicating strain used (DeKlerk and Smit, 2009) 15.5%; Barefoot and Klaenhammer (1983), 81%. Schillinger and Lücke (1989) (23%), out of 221 strains tested, 19 Lb. sake3 Lb. plantarum and 1 Lb. curvatus had an antagonistic action. Rammelsberg and Radler (1990) find out of 79 Lactobacillus only 12 had an inhibiting activity (15%). 36 strains of 100 isolates from traditionally fermented products produce a bacteriocin such as the nisin, 9 of 42 strains of lactic acid bacteria produce bacteriocin.

The presence of inhibition ring does not mean production of bacteriocin inevitably.From the tests, it was necessary to know the exact nature of the inhibiting agent. It may be that inhibition is due to the production of organic acids, hydrogen peroxide, phages and/or bacteriocin (Tagg *etal*., 1976; Barefoot and Klaenhammer, 2012).

The bacterial strains that we isolated are not lysogenic, certain authors announced that the ranges of lyses are not always detectable. Indeed in certain cases, the lysogenic phage exists but does not give ranges of lysis (Chopin *et al*., 1986). When the interaction between the lactic acid bacteria is not due to the bacteriophages, it is caused by the release of molecules as the hydrogen peroxide (Stiles and Holzapfel, 1997; Ross *et al.*, 2002); organic acids or the bacteriocin (Tagg *et al.*, 1976); Barefoot and klaenhammer, 1984). We showed that the inhibition caused by Ln. mesenteroides subsp. dextranicum (LNM04) and *Lb. plantarum* (LPB01) is due to the acid and hydrogen peroxide production. For the 15 strains tested the acid and/or hydrogen peroxide production is responsible for approximately 40% of inhibitions.In addition to these two inhibiting agents, the following strains *Lc. lactis* subsp lactis LCL05, LCL13 and LCL14, *Lc. lactis* subsp lactis biovar diacetylactis LCD04, *Lb. plantarum* LBP02 and LBP03 synthesis a bacteriocin-like.Gilliland and Speck (1977) showed that the addition of catalase in the culture media reduced the inhibition but does not eliminate the production of H_2O_2 with *Lb. acidophilus*. According to those authors, the hydrogen peroxide is partially responsible for antagonism. The antibacterial action produced by *Lb. acidophilus* is probably due to the combination of factors including acidity, hydrogen peroxide and other inhibiting substances. The inhibitory agents produced by the isolated lactic acid bacteria examined in this study could be characterized as bacteriocins-like , since inhibition due to acid, hydrogen peroxide and bacteriophages have been excluded. Also, the proteinaceous nature of the inhibitory substances produced by the strains was confirmed by their protease sensitivity.Some inhibiting substances were characterized as being antimicrobialproteins and called bacteriocins. They should be sensitive to the action of the proteolytic enzymes (Upreti and Hinsdill, 2012; Tagg *etal*., 1976; Barefoot and Klaenhammer, 1983; Klaenhammer, 2012). Some strains produce only proteinic substance which would act like bacteriocin. The response of the culture (solid medium or liquid medium) to the action of proteolytic enzymes is not the same. It is possible that this antagonist agent contains only one substancemade up major of protein nature case of the glycoprotein. In the literature, the found bacteriocins have various reactions with the proteolytic enzymes action.The inhibiting substance produced by Ln. gelidum UAL187 is sensitive to the treatment to the pronase and trypsin (Hastings and Stiles, 2008). *Lb. brevis* produces brevicin 37 whose action is inactivated by the pronase E and trypsin just as the casicin 80 synhesized by *Lb. casei* is sensitive to the protease E and α-chimotrypsin (Rammelsberg and Radler, 1990). The action of the proteolytic enzyme does not raise inhibition completely, of the times we have a reduction in the ring of inhibition. The antimicrobial activity of Lb. plantarum J-51 is lost after treatment with protease (Navaro *et al.*, 2000). Gasserin, a bacteriocin produces by *Lb. gasseri* is sensitive to the action of proteolytic enzymes, resistant to heat like leucocin BC2 and lactocin G13 produces, respectively by Leuconostoc mesenteroides and Lactococcus lactis (Jans *et al*., 1999). Several authors raised the difference between the results of inhibition on solid medium and liquid medium. In the majority of the cases, inhibition is lost in liquid medium. This can be due to several factors; the activity can be lost by filtration through the membrane of 0.2 μm case of the bacteriocin of Pediococcus damnosus B69 (Rammelsberg and Radler, 1990). Schillinger and Lucke (1989) noted the same thing; on 19 strains only 6 presented an activity in liquid medium. The absence of inhibiting activity of the filtrates can be due either to weak concentration of the inhibiting substance (Geis, 1989) or with the loss of the activity after filtration. The resistance of Gram-negativebacteria is attributed to the particular nature of their cellular envelope, the mechanisms of action described for the bacteriocins utilizing an adsorption of these molecules to the sensitive cells. According to Bhunia *et al.* (2008), the pediocin AcH produced by Pc. acidilactici H interacts with the lipotechoïcacids, absent in Gram-negative bacteria. These molecules would play the role of reception site nonspecific necessary to produce the bactericidal effect. Bhunia *et al*. (2008) assign the resistance of the gram-negative bacteria to the pediocin AcH to the barrier which their external membrane would represent.The incapacity of the bacteriocins to cross this barrier is due totheir molecular weight and/or their hydrophobic properties. In the case or the externalmembrane is made permeable, either by a physical treatment (Kalchayanand *et al*., 1992) or by a chemical treatment, the gram-negative bacteria become sensitiveto the bacteriocins.

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