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Selection of lactic acid bacteria producing antimicrobial strain such the genus lactococcus isolated from algerian raw goat's milk

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Abstract: Lactic acid bacteria are known for their ability to produce inhibitory substances against unwanted germs involved in food poisoning such as Listeria innocua. Species of lactic acid bacteria can inhibit harmful germs subject of this work. techniques confrontation on solid medium and the effect of the substance on the growth of Listeria innocua were performed. The experience was conducted bacterial antagonism in the solid medium by the method of double layer and method of wells, the whole experience was packaged in a buffered medium in order to eliminate the effect of acidity . 7 strains that isolates gave an antagonistic action against Listeria innocua. The strain of Lactococcus lactis (8b), isolated from raw goat's milk showed an anti-listeria in vitro and was selected because of its ability to inhibit as Staphylococcus aureus. With a diameter of 15mm, the action of proteolytic enzymes, trypsin, chymotrypsin is shown that the substance was protein in nature, the kinetics of growth in milk medium showed that the number of St. aureus after 24 h of incubation was reduced to 7.68 log cfu in mixed culture with strain 8b which was comparable to the control of 9.14 log cfu, which signifies that the survival rate was 3.4%, the phenotypic (biochemical and physiological) and molecular-based ADNr16Ss showed that the strain is Enterococcus faecium, duran, hirae. The sequencing results showed that Leuconostoc mesenteroides and Enterococcus faecium have summers dominant anti-Listeria species in milk samples from Algerian goat. Isolates had the potential of multiple bacteriocin production and do not have some important virulence. Importance and impact of the study: The Enterococci in milk in this region of western Algeria could be partly responsible for the safety of cheese and could be useful for the production of anti-Listeria cultures protection. The purpose of the study: Our study's main objective is the selection ,and study phenotypic and molecular lactic acid bacteria from Lactococcus genus possessing biotech traits such as the production of new inhibitory substance and the study of the interaction of Listeria innocua screw opinion and St. aureus that both methods were used, a traditional approach based on morphological and biochemical studies of different cultural characteristics and a method of direct molecular amplification of bacterial DNA using PCR / RAPD colony on having targeted DNA fragment 16S lactic acid bacteria using the two primers universal: 20F (5'AGAGTTTGATCATGGCTCAG-3 '). 1500R (5'-GGTTACCTTGTTACGACTT-3 ') and two specific primers OPA-3 (5'-AGTCAGCCAC - 3') and OPH 3 (5'-AGACGTCCAC-3 ') (Bioprobe, France).

Keywords: Bacteriocin, Listeria, St. Aureus, Goat's Milk; Adnr16s, PCR / RAPD

1. Introduction

Lactic acid bacteria possess remarkable technological advantages for bio food preservation (Ananou.S, 2010, Kouakou Privat, Philippe Thonart2011), thanks to the production of a wide variety of antimicrobial substances (compounds antagonists) which prevent the growth of pathogens transmitted by food (Listeria, Staphylococcus, (Labioui., 2005, Gravesen A et al 2011). The main factor responsible for the inhibition in fermented foods is the production of organic acids and the concomitant decrease in pH. Moreover, lactic acid bacteria can secrete inhibitors in the middle specific and which are the most interesting technological standpoint due to their nature protein (Thonart and Dortu, 2009). inhibitors are known bacteriocin. Lactic

acid bacteria produce a variety of peptides or proteins having activity antibacterial. called bacteriocins These molecules could be better used in dairy various transformations, notably to ensure the hygienic safety of certain cheeses. (Bayoub et al., 2006 Dortu, 2008). bacteriocins represent a large class of antagonistic substances which vary considerably in terms of their molecular weight, their biochemical properties, their spectrum and their mode of action (Dortu 2008). All bacteriocins produced by lactic acid bacteria described so far have activity against Gram-positive bacteria. however, the modes of action of bacteriocins on the membrane are varied (Dortu, 2008). Currently a new generation of vaccine is being developed using strains of lactic acid bacteria (Touvart 2009, M.Zadeh 2009).

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions

Lactococcus lactis subsp lactis was isolated from raw goat milk from Algeria. Stored at -20 ° C in a medium containing 10% milk (0.05% enriched yeast extract) and 30% glycerol deposited at a temperature of (-20 ° C). As the need arises, the cultures are thawed quickly in milk and subcultured twice before use (Samelis et al, 1998). St. aureus, Listeria, Bacillus and E. coli were stored at -20 ° passaged several times in the liquid medium at 37 ° C soybean strain was subcultured inhibitory several times in MRS medium and M17 liquid at 30 ° C.

Listeria innocua strain (S71 and LMBA St.aureus CIP20256) of ENITA Bordeaux, St. aureus (ATCC.602 laboratory Tlemcen). the research activity inter bacterial antagonist interaction was performed by the direct method: according to Fleming et al., (1975), is to cultivate both strains in the same medium double layer and the indirect method described by Barefoot and al. (1983), that use the supernatant of the culture medium of a bacterium assumed to be inhibitory. The action of the supernatant obtained after centrifugation of the culture medium is tested against a bacterium called sensitive indicator. St. aureus strains, Bacillus Listeria and E. coli were used in both cases come indicator strains. Acidity factor was removed by the use of a buffered medium (0.2 M phosphate buffer pH 7). To detect the production of H2O2, is carried out in the presence of crop in an amount of catalase 1mg/ml medium. The enzyme and the indicator strain were mixed in soft agar supercooled. After incubation reading the results is compared to the control containing no catalase.

2.2. Search Lysogenic Phages

With a Pasteur pipette was cut a piece of agar in the zone of inhibition.

This fragment was suspended in 1 ml of sterile medium containing 50 mu.l of chloroform. After stirring, allowed to settle 5 minutes, then was taken 300µl of medium that is was added to 7 ml of soft agar containing the indicator strain. The mixture was poured aseptically into a petri dish and incubated for 48 h at 28 ° C. The presence of lysis plaque indicates the presence of phage.

2.3. Search Inhibitions Diffusion in Solid

Filtrate was concentrated to 100µl of acetone are placed in wells made previously in M17 medium buffered solid. Deposits are made as follows: Well 1: control (untreated float) Well 2: + trypsin filtrate, Well 3 + filtrate chymotrypsin, Wells 4 + filtrate lysozyme, Well 5: filtrate heated to 100 ° C for 30 min., Wells 6 filtrate pH 7, Well 7: concentrated filtrate with chloroform.

After a one hour incubation at room temperature, allowing the diffusion potential antimicrobial substances. Petrie boxes are covered with 7 ml of soft agar containing the indicator strain. : Following a second incubation of 24 hours.

2.4. Growth Kinetic and Acidification

The evaluation of the acidity produced by a pure strains was performed by titration and pH - metritis. Each strain was inoculated into 10ml sterile skim milk. Following incubation until coagulation of milk at 28 ° C for 18 h or more (there was clotting time), everything is transferred into sterile 200ml skimmed milk and homogenized. the mixture was distributed into sterile tubes due 10ml/tube and the kinetics of growth and acidification were performed simultaneously have the following time intervals: 0h, 3h, 6h, 9h, 14h, 16h, 24h, 48h, 72h, 96h. For this study we used a strain of lactic acid bacteria strain as inhibitory considered effective strain and a pathogenic strain as Staphylococcus aureus strain test laboratory of Tlemcen ATCC.602 that the reason inoculate 103 - 105 cfu / ml and according Hamama al., 2003), we calculated the evolution of a count from 0 h after 18 h preculture of each strain and strain inhibitory test and mixed culture.

2.5. Random Amplified Polymorphic DNA (RAPD)

The random amplified polymorphic deoxyribonucleic acid (Random Amplified Polymorphic DNA) is performed directly on colony strains étudiées.Cette amplification of DNAs is performed in a thermal cycler (PTC-200, MJ Research, USA) with the program starting with a initial denaturation step at 94 ° C durant5 min, followed by 45 cycles of amplification of the DNAs with three stages that are 30 s at 94 ° C, 1 min 30 s at 36 ° C and 1 min 30 s at 72 ° C. Immediately after the last cycle of amplification, the reaction mixture was kept at 72 ° C for 10 min to complete the amplification possible. DNA fragments amplified by PCR were then separated by electrophoresis voltage 5 V.cm-1 agarose gel (1.5% [w / v] added BET [ethidium bromide] to reason of 0.1 mg per 300 mL of agarose gel) and then immersed in TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA). Amplification without DNA (negative control) is carried out in each series to control possible contamination of foreign DNAs in the matrix. Reading and photography electrophoretic profiles are performed under ultra violet (UV) transilluminator with (Vilber Lourmat, Allemange) at 254 nm. The

size of the DNA fragments was estimated by comparison with standard DNA marker λ EcoRI / HindIII Digest (Q-biogenic) (Appendix 2). Polymorphic electrophoretic profiles are exploited by comparing the DNA bands on agarose gel. Twenty microliters of the PCR product are sent to Genome Express (France) for sequencing.

The sequences are realized in the genome called Express began, and it is as follows: partial DNA sequences of the PCR products (the first 600 bases) were determined using a Taq Dye Deoxy terminator sequence kit cycle (Perkin-Elmer, Foster City, CA, USA) and the protocol recommended by the supplier. Products of the sequencing reaction were analyzed with a model 373A automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Databases (GenBank) looked for sequences similar to the 16S rDNA sequence obtained.

3. Results and Discussion

Among the 48 strains confrontations between 768 and according to the percentage (%) inhibition was able to get 460 cases or 60% inhibition. These results were obtained using a medium is not buffered. In contrast, there was a small number, 130 cases of inhibition, in the middle 16% buffered. Of the 48 strains, 36 strains possess an inhibitory power and 12 strains showed no inhibition in buffered medium (see Table 1).

According to the percentage of inhibition is buffered medium 20 strains were selected (16 and 4 inhibitory indicator strains (see Table 2).

Table 2. *Percentage (%) inhibition of various confrontations OF inhibitory and indicator*

CODE	(%) d'inhibition Série 1		(%) d'inhibition Série 2		(%) d'inhibition Série 3	
Inhibitrice	Ds53	42%	18b	18%	2b	18%
Inhibitrice	12b	42%	T16	48%	20b	42%
Inhibitrice	11a	42%	1b	72%	Ds15	42%
Inhibitrice	Ds16	36%			8b	24%
Inhibitrice	7b	30%			30a	72%
inhibitrice	4a	42%			17a	34%
Inhibitrice					Ds13	24%
indicatrice	24a	42%	T20	54%		
Indicatrice			Ds44	42%		
indicatrice			Ds14	24%		

Table 1. *percentage inhibition (INH) inter bacterial environment Buffered (MT) and unbuffered (MNT) for 16 strains by the method directly.*

	Code des souches	INH/16 MNT	INH/16 MT	% MNT	% MT
Série 1	7b	16	5	100	30
	11a	16	7	100	42
	7a	16	1	100	6
	Ds53	16	7	100	42
	Ds16	14	6	84	36
	12b	15	7	90	42
	4a	15	7	90	42
	3a	15	1	90	6
	21a	16	0	100	0
	18a	15	1	90	6
	Ds8	0	0	0	0
	25a	15	1	90	6
	24a	16	0	100	0
	Ds44	16	0	100	0
	35T	15	0	90	0
	34b	15	2	90	12
	Ds14	1	0	6	0
	22a	11	2	66	12
	T16	11	8	66	48
	T20	11	2	66	12
	6a	10	1	60	6
	Ds36	0	0	0	0
	11b	0	0	0	0
Série 2	Ds37	11	1	66	6
	18b	13	3	98	18
	T35	13	0	96	0
	Ds39	0	1	0	6
	1b	11	12	66	72
	Ds18	7	2	42	12
	Ds51	0	0	0	0
	31b	4	2	24	12
	25b	10	0	60	0
	Ds13	12	4	72	24
	Ds29	0	1	0	6
	29b	11	2	66	12
	27b	5	1	30	6
	28b	13	2	78	12
	Ds52	15	1	90	6
	17a	0	4	0	34
Série 3	16a	15	0	90	0
	Ds15	1	7	6	42
	8b	13	4	78	24
	19a	0	1	0	6
	30a	12	12	72	72
	2b	9	3	54	18
	T24	12	1	72	6
	20b	13	7	78	42
	Ds23	0	2	0	12

The 20 strains that were selected were reidentified by physiological and biochemical testes (see Table3).

Table 3. *Physiological and biochemical character strains*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Leuconostoc mesenteroides subsp.mesenteroides	2b	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Lactococcus lactis sub sp lactis biovar diacetylactis	1b	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis biovar diacetylactis	20b	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis biovar diacetylactis	Ds 15	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis	8b	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis biovar diacetylactis	30a	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis	4a	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis	Ds 13	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Streptococus thermophilus	12b	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	+	-	+	-
Streptococus thermophilus	11a	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	-	-
Lactococcus lactis sub sp lactis	Ds 16	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Enterococcus sp	7b	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
Lactococcus lactis sub sp lactis	T1 6	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Enterococcus sp	17a	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
Streptococus thermophilus	18b	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+	+	-	+	-
Lactococcus lactis sub sp lactis	Ds 53	+	-	-	-	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Lactococcus lactis sub sp lactis biovar diacetylactis	24a	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	-	+	-	-
Leuconostoc mesenteroides subsp.mesenteroides	T2 0	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
Pediococcus acidilacticii	Ds 44	+	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-
Lactococcus lactis sub sp lactis	Ds 14	+	-	-	-	+	+	+	+	-	+	+	+	-	-	+	-	+	+	+

1=code of strain,2=Gram,3=catalase,4=citrate,5=acetoine,6=Arginine,7=dextrane,8=croissance àpH(9.2),9=croissanceNacl(4%),10=croissanceNacl(6,5%),11=Glucose,12=Lactose,13=Galactose,14=Maltose,15=Manitol,16=Raffinose,17=Saccarose,18=Xylose,19=Arabinose,20=Production du gaz.

Table 4. *Interaction between bacteria Gram + and Gram-*

Nom espèce	Code	St. aureus ATCC.602(TLEMCEN)	St.aureus Cip20256(BORDEAUX)	Listeria inoccua		E.coli	Bacillus cereus	24a indicatrice
		Diamètre de la zone d'inhibition (mm)						
		MRS	MRS	MRS	M17	MRS	MRS	MRS
Leuconostoc mesenteroides subsp.mesenteroides	2b	05	0	-	-	0	0	08
Lactococcus lactis sub sp lactis biovar diacetylactis	1b	11	0	12	10	0	0	12
Lactococcus lacti s sub sp lactis biovar diacetylactis	20b	12	0	13	11	0	0	14
Lactococcus lactis sub sp lactis biovar diacetylactis	Ds15	04	0	-	-	0	0	03
Lactococcus lactis sub sp lactis	8b	15	0	14	11	0	0	13
Lactococcus lactis sub sp lactis biovar diacetylactis	30a	08	0	-	-	0	0	11
Lactococcus lactis sub sp lactis	4a	10	0	12	10	0	0	11

The results of the interaction vis the advice of St. aureus and Listeria results show that among the 16 strains inhibit 7souches St.aureus (Cip20256 Bordeaux) (see fig3) strain and 10 inhibit St.aureus ATCC.602 (Tlemcen), (see fig2) strain 8b has a diameter of 15mm, this strain was tested vis-à-vis listeria she gave a 14mm diameter wells by the method of the indirect method described by Barefoot et al. (1983), that use the supernatant of the culture medium of a bacterium assumed to be inhibitory. The action of the supernatant obtained after centrifugation of the culture medium is tested against a bacterium called sensitive indicator (see fig4, Table 4).

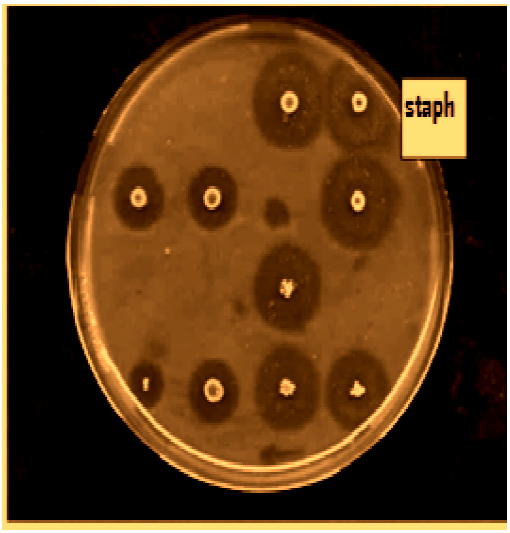

Fig.2. *Interaction between de isolates and St.aureus ATCC.602(tlemcen)*

Fig.3. *Interaction between de isolates and St.aureus (Cip20256 Bordeaux)*

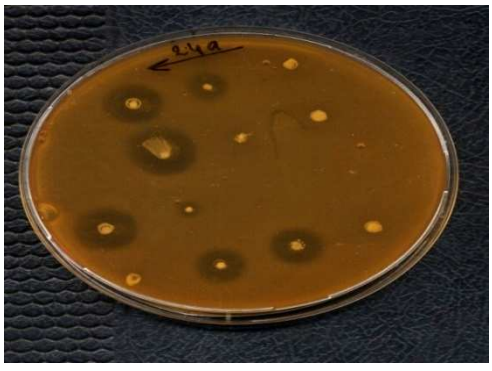

Fig.4. *Interaction between isolates and indicator strain 24a*

Strains have shown a large diameter screw the advice of St. aureus, 1b, 20b, 8b, 4a were tested agains listerie innocua.

The results show that the supernatant adjusted pH7pour strains 8b, 20b, 1b and 4a gave inhibitions vis-à-vis Listeria innocua after 24 h of incubation at 30 ° (see fig5)

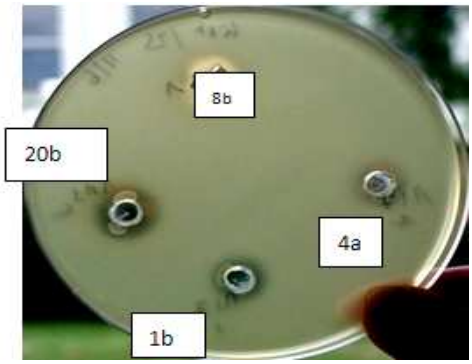

***Fig.5**. interaction between isolates and de listeria innocua*

Determining the nature of the inhibitor: The results showed that among 48 strains tested after confrontation 768 7 strains have been selected 1b strains, 8b and 30a are presented inhibitions vis-à-vis the test strain with a diameter of 14 and 15mm. (Untreated supernatant) they are noted sensitivity vis-a-vis the trypsin and alpha chymotrypsin by cons. strains 2b, 20b, DS15 and 4a is shown resistance vis-à-vis the proteolytic enzymes.

***Table** 5. Highlight interaction between lactic acid bacteria in the presence and absence of proteolytic enzyme*

Nom espèce	Code	Puits vide	Trypsine	Achy Motripsine	Lysozyme	100°	pH7	Chloro-forme
Leuconostoc mesenteroides subsp.mesenteroides	2b	1,2	0,2	0,2	0	1,3	1,3	1,1
Lactococcus lactis sub sp lactis biovar diacetylactis	1b	1,5	0	0	0	1,1	1,3	1,2
Lactococcus lactis sub sp lactis biovar diacetylactis	20b	1,3	0,1	0,1	0	1,3	1,4	1,1
Lactococcus lactis sub sp lactis biovar diacetylactis	Ds15	1,2	0,1	0,1	0	1,2	1,4	1,2
Actococcus lactis sub sp lacts	8b	1,5	0	0	0,2	1,2	1,5	1,2
Lactococcus lactis sub sp lactis biovar diacetylactis	30a	1,4	0	0	0	1,2	1,4	1,3
Lactococcus lactis sub sp lactis	4a	1,2	0,2	0,2	0	1,2	1,4	1,3

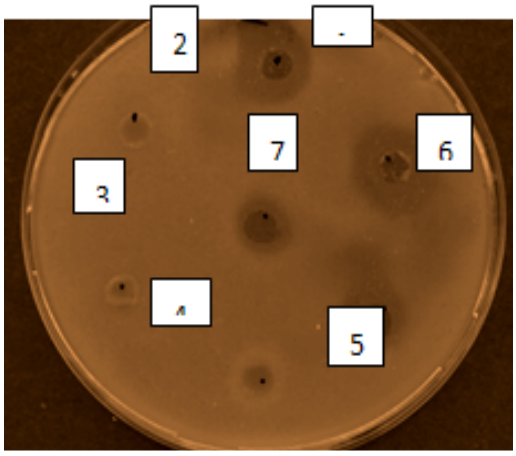

***Fig.1.** Effect of the nature of the inhibitor*

1-Filtrat non traité 2-Filtrat +trypsine 3-filtrat+αchymotrypsine 4-Filtrat+lysozyme 5-filtrat chauffée à 100° 6-Filtrat ajuster à pH=7 7-Filtrat + chloroforme

All strains are presented for the inhibition test supernatant heated to 100 °, and the supernatant adjusted to pH = 7. and the supernatant added chloroform.(See table5, fig 1).

For the study of the kinetics of acidificationet grows we chose the most efficient strain 8b aprésenté diameter 15mm agains St.aureus and 14mm screws to screw listeria.

After 24 h of incubation, the pH of the producing strain 8b could reach 4.53 and 5.2 for Staphylococcus aureus. An antagonism was detected in mixed culture and the pH was always equal to 24 hours so it has 6 results confirmed that there was a conflict between the producer strain and strain test (see Table 6 fig6) Same results were observed by (Hamama et al, 2002), pH = 4.50 after 24 h of incubation, 4.23 after 72 h of incubation pH = 4.14 after 96h incubation the amount of lactic acid produced by pure cultures did not show a big difference 4.7 g for the producing strain (8b) and 3g for Staphylococcus aureus after 24 h of incubation.

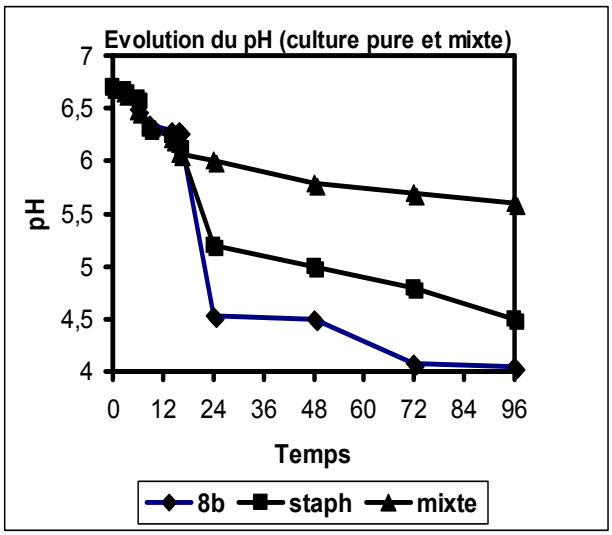

***Fig.6**. Evolution of pH as a function of time*

***Table 6**. Evolution of pH as a function of time*

Temps (h)	8b	St.aureus	Culture Mixte
0	6.71	6.70	6.71
3	6.66	6.67	6.65
6	6.48	6.59	6.47
9	6.35	6.30	6.34
14	6.29	6.24	6.20
16	6.28	6.15	6.06
24	4.53	5.2	6.00
48	4.50	5.0	5.78
72	4.07	4.8	5.70
96	4.05	4.5	5.60

For the mixed culture showed a decrease in the amount of lactic acid 50% which means that there is an antagonism between the two strains. (See array7, fig7).

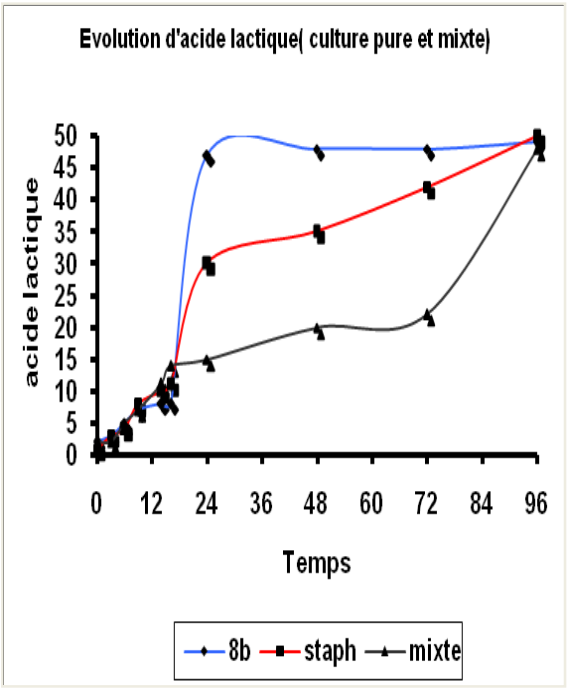

***Fig.7**. Evolution of lactic acid as a function of time*

***Table 7**. Evolution of lactic acid as a function of time*

	8b	St.aureus	Mixed culture
Temps(h)	Lactic acid (g /l)		
0	2	1	1
3	3	3	3
6	5	4	5
9	7	8	7
14	8	10	11
16	8	11	14
24	47	30	15
48	48	33	20
72	48	42	22
96	49	50	48

These results confirm what has been demonstrated by Hamama et al. (2002) in mixed culture the amount of lactic acid produced after 18 h preculture was 1.13g after 24 hours of incubation and it was after 24 1.5g h incubation.

For the kinetics of growth in pure culture was not observed a significant difference in the number of log unit. After 24 h of incubation, the number of logarithmic unit for producing strain 8b was almost like her either in pure culture (10.28 log cfu g-1). Or mixed (10.77 log cfu g-1 for the test strain Staphylococcus aureus the case was different, it was observed a decrease of strain in pure culture was 9.14 log cfu g-1 and 7.68 cfu g-1 in mixed culture after 24 h of incubation. (see Table 8 fig8).

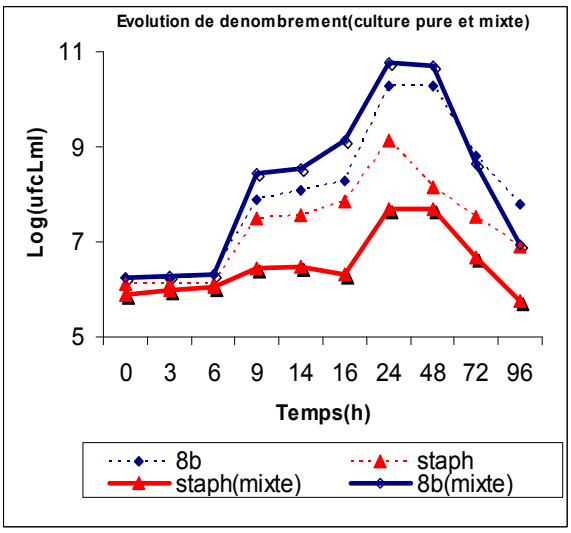

***Fig.8**. Evolution of the counting function of time. M 1 2 3 4 5 6 7 8 9 10 1 12.*

Table 8. *Evolution of dénombrement as a function of time*

Temps(h)	8b (MRS)	St.aureus (chapman)	St.aureus(Mixte) (Chapman)	8b(Mixte) MRS
0	6.24	6.10	5.87	6.25
3	6.20	6.11	5.99	6.28
6	6.30	6.13	6.04	6.31
9	7.90	7.48	6.43	8.44
14	8.08	7.55	6.47	8.53
16	8.27	7.86	6.30	9.12
24	10.28	9.14	7.68	10.77
48	10.27	8.14	7.68	10.71
72	8.81	7.51	6.68	8.65
96	7.79	6.90	5.74	6.92

The growth rate of $\mu = 0.73$ for $\mu = 0.59$ 8b strain to strain St. aureus and 0.17 for mixed culture.

The result of the random amplified polymorphic deoxyribonucleic acid (Random Amplified Polymorphic DNA) on colony using specific primers as 0PA3 allowed us to detect DNA bands corresponding to ADNr16s discomfort among strains studied, all amplicons (7a, 15a, Ma27, DS16, 1b, 20b and 30a have the same electrophoretic profile corresponding to a DNA fragment is a 1.5 Kbp (see fig9). and for all primer OPH3 emplimères 18a, 27b, DS29, 25a, DS52, 4a, DS15 has a similar profile électroforètique corresponds to a DNA fragment located at (1.5 kbp) (see Figure 10).

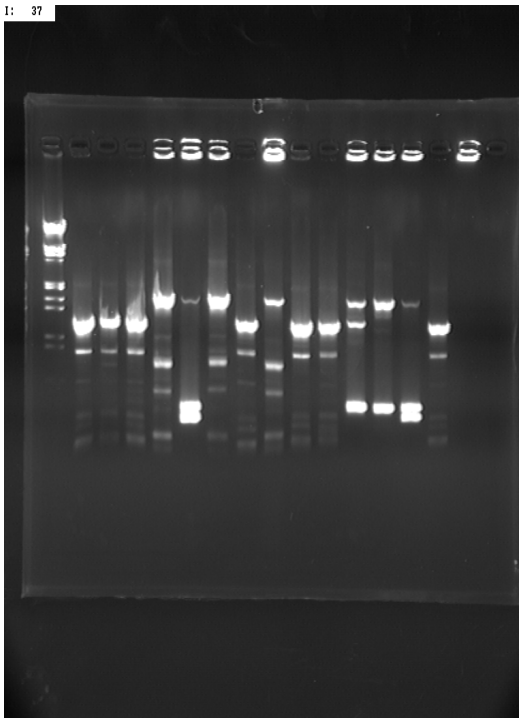

Fig.9*. Following amplification assay analyzed by electrophoresis agarose gel of different strains of Lactococcus (primer OPA3). M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16*

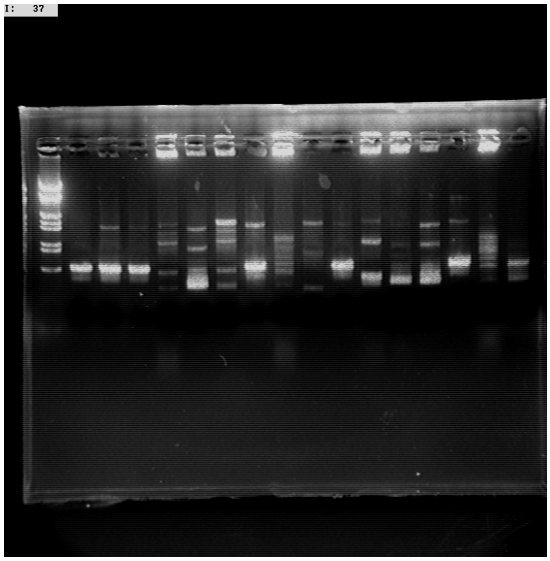

Fig.10. *Following amplification assay analyzed by electrophoresis agarose gel of different strains of Lactococcus(primer OPH3). M 16 17 18 19 20 21 22 23 24*

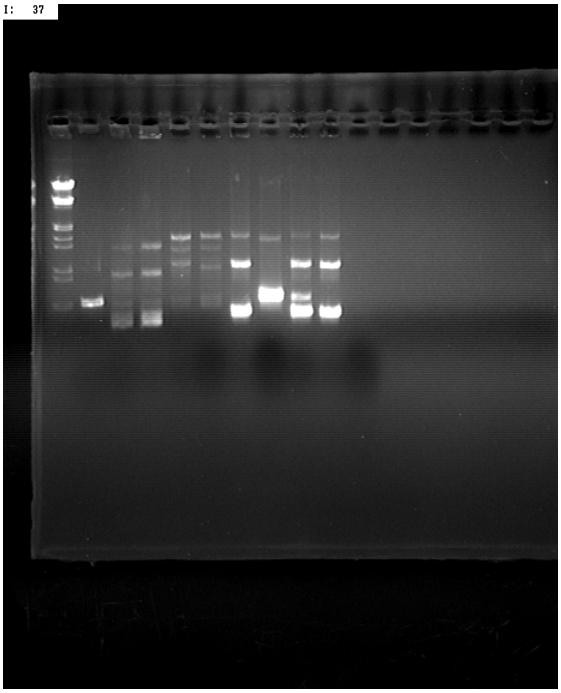

Fig.11. *Following amplification assay analyzed by electrophoresis agarose gel respectful strains of the genus Enterococcus and Streptococcus and Pediococcus termophylus (primer OPA3)*

These results are comparable to those found by Hamama et al, (2002) who found that the number of logarithmic unit of Staphylococcus aureus was 5.36 after 24 h of incubation in mixed culture, whereas this number was 7.68 after 24 h incubation.

According to the literature the size of the gene coding for 16S rRNA in a measure lactococus 1540pb (Roudidre L et al, 2007) confirming that the bands represented in the electrophoretic profile correspond to fragment ADNr16s therefore studied strains belong all the kind of lactococus.

4. Conclusions

Phenotypic methods may not be the final result of the identification of the bacterial species random amplification of DNA polymorfisme (RAPD) sequencing revealed 12 strains of Leuconostoc mesenteroides, 8 strains Enterococcus faecium, duran, and 4 St.thermophilus (see Table 9). These results are comparable with those found by CHANOS et al 2011 that enterococci were the most dominant in the milk medium.

Table 9. *Phenotypic and molecular identification of isolates*

N°	code	Identification phénotypique	Séquençage PCR/RAPD
1	7a	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
2	15a	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
3	Ma27	L. lactis sub sp.lactis	Leuconostoc mesenteroides
4	18a	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
5	27b	L. lactis sub sp.lactisbiovardiacetylactis	Enterococcus faecium, durans, hirae
6	Ds29	L. lactis sub sp.lactis	Leuconostoc mesenteroides
7	Ds16	L. lactis sub sp.lactis	Leuconostoc mesenteroides
8	25a	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
9	1b	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
10	20b	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
11	Ds23	L. lactis sub sp.lactis	Leuconostoc mesenteroides
12	4a	L. lactis sub sp.lactis	Leuconostoc mesenteroides
13	Ds15	L. lactis sub sp.lactisbiovardiacetylactis	Enterococcus faecium, durans, hirae
14	30a	L. lactis sub sp.lactisbiovardiacetylactis	Leuconostoc mesenteroides
15	Ds53	L. lactis sub sp.lactis	Enterococcus faecium, durans, hirae
16	Ds52	Pediococcus acidilacticci	Streptococcus thermophylus
17	8b	Enterococcus sp.	Enterococcus faecium, durans, hirae
18	17a	Enterococcus sp.	Enterococcus faecium, durans, hirae
19	21a	Enterococcus sp.	Streptococcus thermophylus
20	6a	Enterococcus sp.	Streptococcus thermophylus
21	7b	Enterococcus sp.	Enterococcus faecium, durans, hirae
22	31b	Streptococcus thermophylus	Streptococcus thermophylus
23	11a	Streptococcus thermophylus	Enterococcus faecium, durans, hirae
24	18b	Streptococcus thermophylus	Enterococcus faecium, durans, hirae

Leuconostoc mesenteroides and Enterococcus faecium have summers dominant anti-Listeria species in milk samples from the western Algerian goat. Isolates had the potential of multiple bacteriocin production and do not have some important virulence.

Importance and impact of the study: The Enterococci in milk in this region of western Algeria could be partly responsible for the safety of cheese and could be useful for the production of anti-Listeria cultures protection.

The selection of strains that are potent inhibitors was based on two points: the diameter of the zone of inhibition on solid medium and the percentage of inhibition in mixed culture.

The statistical results based on student test confirmed that our results were significant confirming that the buffered medium is favorable medium for the detection of inhibitory strains. Interactions with Staphylococcus aureus showed that seven strains possess an inhibitory effect.

Strain 8b gave considerable inhibition zones agains Listeria innocua and Staphylococcus aureus, the inhibition is due to an inhibitory substance knowing that the whole experience has been packaged in a buffered medium. Additional tests peroxide namely, phage, and the effect of proteolytic enzymes confirmed that the protein nature of this inhibitory substance which is a bacteriocin as defined characters Tagg et al (1976).

Phenotypic methods can not be the final result of the identification of the bacterial species random amplification of DNA polymorfisme (RAPD) sequencing revealed 12 strains of Leuconostoc mesenteroides, 8 strains Enterococcus faecium, duran, and 4 strain of St.thermophilus. These results are comparable with those found by CHANOS et al 2011 that enterococci were the most dominant in the milk medium. Importance and impact of the study: The Enterococci in milk in this region of western Algeria could be partly responsible for the safety of cheese and could be useful for the production of anti-Listeria cultures protection. Leuconostoc mesenteroides and Enterococcus faecium have summers dominant anti-Listeria species in milk samples from goats. Isolates had the potential of multiple bacteriocin production and do not have some important virulence.

Lactic acid bacteria behave as excellent ambassadors of the microbial world often maligned. They can not be reduced to their economic importance, but play an important role in the maintenance and improvement of human health.

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