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Isolation and Identification of Lactic Acid Bacteria from Dhan, A Traditional Butter and Their Major Technological Traits

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Abstract: A total of 5 samples of traditional fermented milk butter (Dhan) were collected from individual households. Lactic acid bacteria dominated the microflora of these samples, especially the genera *Leuconostoc*, *Lactococcus* and *Lactobacillus*. Other groups identified included pyogenic streptococci and enterococci. The dominant *Lactococcus* species was *Lactococcus lactis* subsp. lactis. Eighty-three percent of the *Leuconostoc* isolates were identified as *Leuconostoc mesenteroides* subsp. *dextranicum*. Other species identified included *Leuconostoc citreum*, *Leuconostoc lactis*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus plantarum*.

Key words: Lactic Acid Bacteria · *Lactobacillus* · Dhan · Traditional Fermented Milk butter · Households

INTRODUCTION

Lactic acid bacteria (LAB) have played along an important role in food technology and have a long history of use by man for food production and food preservation. Isolation of wild-type strains from traditional products is a classical method to obtain starter cultures for food fermentations [1]. By using selected wild-type strains, the large-scale production of fermented foods can be developed without losing their unique flavor and particular characteristics [2].

The samples used in this study are traditional butter milk made from unpasteurized cow milk. First, milk is left at room temperature until it tastes sour. A manual agitation is operated in a goatskin churn, during agitation a small amount of cool water is added. This cool water helps to coagulate fat globules. This agitation lasts a few tens of minutes and fat contents are collected representing butter. Thus butter obtained is salted and it should be noted that salting is to leave with the taste of sparing. The butter is preserved at room temperature and rancidity will take place after a few days.

Numerous researches demonstrated that the autochthonous microflora present in several traditional products, other than improving the final technological and sensory characteristics, possess inhibition activity towards spoilage and pathogenic bacteria and this has been reported for both dairy [3-6] and meat products [7-8]

The aims of this study were: i) to study the microbial ecology of lactic acid bacteria of Dhan [traditional butter milk] and ii) characterization of different groups of microflora, lipolytic, proteolytic and antimicrobial producing bacteria using classical methods.

MATERIALS AND METHODS

Collection of Samples: Five samples of traditionally butter milk (dhan) where purchased from local households in west Algeria. Samples were transported to the Laboratoire de Microbiologie Appliquée in room temperature.

Isolation and Identification of Lactic Acid Bacteria: Ten grams of butter was homogenized with 90 mL sterile NaCl solution (0.85%, w/v) to a homogenous suspension and then a tenfold serial dilution in NaCl solution (0.85, w/v) was carried out. For isolation of lactic acid bacteria, acidified MRS pH 5.4 [9] incubated anaerobically for 72 h at 37°C was used for isolation of lactobacilli. MSE medium [10] incubated aerobically for 48 h at 35°C was used for isolation of leuconostocs. M17 agar [11] incubated aerobically for 48 h at 30°C for the isolation of lactococci.

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The colonies between 30 and 300 on each Petri dish were counted as total LAB. To isolate LABs, ten colonies were randomly picked from each countable plate. Attention was given to choose colonies with different macroscopic morphology. Isolates were re inoculated into MRS broth, incubated at 30°C and checked for purity by streaking on MRS agar. Plates with pure cultures were used to test for cell morphology by phase contrast microscopy, Gram stain and catalase formation. Gram positive and catalase negative strains were selected. These isolates were maintained as frozen stocks in MRS broth supplemented with 10% (v/v). Before experimental use, all LAB strains were recovered in MRS broth and incubated at 30°C.

Growth at different temperatures was observed in MRS broth after incubation for 5 days at 15°C and 45°C. Supplemented test was performed for lactococci and resistance at 63.5°C for 30 min was done in order to discard enterococcus bacteria [12]. Hydrolysis of arginine was tested in M16BPC [13, 14]. Growth in the presence of 4 and 6.5% NaCl was performed in MRS broth for 5 days. Utilization of citrate was realized in Kempler and Mc Kay [15] medium. Production of acetone from glucose was determined using Voges-Proskauer's test [16]. Dextran production from sucrose was done in MRS agar [10] and CO_2 gas production in Durham tube within MRS broth was checked from glucose.

The sugar profile of each strain was determined in mini preparation with ELISA microtiter plate [17], with MRS broth without lactose and meat extract adjusted to pH 6.5 as fermentation medium, with bromocresol purple [0.004%] as indicator [MRS-BCP]. Individual sugars (Sigma Chemical Co., St. Louis, MO, USA) were sterilized by filtration and added to a final concentration of 2%. Microbial cells were washed and added in a sterile saline solution (8 g NaCl/L). Each ELISA microtiter plate well, received 0.1 ml MRS-BCP medium, 10 µl microbial cells and 10 µl sugar solution. 0.1 ml sterilized paraffin oil was added and the incubation was made at 30°C and the results were read after 1 and 2 days of incubation.

Lipolytic Activity of Isolated Microorganisms: The culture medium was prepared by adding peptone 10.0g, NaCl 5.0g, $CaCl_2 2H_2O$ 0.1g and agar 20.0 g in 1000ml water and autoclaved for 20 min; 10ml Tween-20 [Sigma] was separately sterilized and added to the autoclaved medium and the pH was adjusted to 6.0. About 20ml the medium was poured into each Petri dish and inoculated at the center using a pinpoint inoculum of the test isolate. Lipolytic activity was indicated by the appearance of a visible precipitate, resulting from the deposition of crystals of the calcium salt formed by the fatty acid liberated by the enzyme, or as a clearing of such a precipitate around a colony due to complete degradation of the salt of the fatty acid. At regular intervals of 24 h incubation, each plate was examined and measurements were taken to monitor lipolytic activity [18-20].

Proteolytic Activity of Isolated Microorganisms: Proteolytic activity was tested using plat count agar (PCA) with 1 and 2 % [w/v] skimmed milk. The presence of clear zones around the colonies was recorded as positive activity. All strain with positive reaction in MRS with 1% skimmed milk was considered as strains with slight activity [21].

Screening for Antagonistic Activity: Detection of antagonistic activity of LAB strains was initially screened by means of an agar well diffusion assay (AWDA) [22]. MRS agar was used for LAB strains while BHI agar was used for the rest of the indicator microorganisms (*Staphylococcus aureus, Escherichia coli* and *Listeria innocua*). Briefly, Petri dishes were overlaid with 15 ml of molten agar (1% agar), inoculated with 30 µl of an overnight culture of the indicator microorganism, in which wells of 5 mm diameter and 30 µl in capacity, were formed. Afterwards, 30 µl of an overnight culture of the putative inhibitor strain were placed in each well. The plates were then incubated aerobically for 24h at a temperature conductive to growth of the indicator microorganism and were subsequently examined for zones of inhibition. Inhibition was recorded as negative if no zone was observed around the agar well. Each antagonistic activity was related to the area of the inhibition zone displayed [23].

Antibiotic Susceptibility Testing: For the antibiogram of isolated lactic acid bacteria strains, the isolates were inoculated into MRS broth individually and incubated for 24 h. About 20 ml of Mueller-Hinton agar was seeded with the cultures of LAB isolates, mixed well, poured into sterile Petri plates and leave to solidify at room temperature. Antibiotic discs (OXOID) (Table 1) were placed up side down and pressed on the top of the agar plates. The plates were incubated at 37ºC over night. Resistance was defined as the absence of a growth inhibition zone around the discs.

Table 1: List of tested antibiotics against isolated lactic acid bacteria

Antibiotic	Concentration	Symbol	Antibiotic	Concentration	Symbol
Amikacin	30 µg	AN	Lincomycin	15 µg	L
Amoxycillin + Clavulanic Acid	20 µg + 10 µg	AMC	Nalidixic Acid	30 µg	NA
Ampicillin	10 µg	AM	Netilmicin	30 µg	NET
Bacitracin	0,02 à 0,04 IU	BAC	Nitrofurantoin	300µg	FT
Cefazolin	30 µg	CZ	Ofloxacin	5 µg	OFX
Cefotaxime	30 µg	CTX	Oxacillin	1 µg	OX1
Cefoxitin	30 µg	FOX	Pefloxacin	5 µg	PEF
Cefsulodin	30 µg	CFS	Penicillin	6 µg / 10 IU	P
Ceftazidime	30 µg	CAZ	Piperacillin	75 µg	PIP 75
Cephalothin	30 µg	CF	Pristinamycin	15 µg	PT
Ciprofloxacin	5 µg	CIP	Rifampin	30 µg	RA 30
Clindamycin	2 µg	CM	Spiramycin	100 µg	SP
Colistin	50 µg	CS 50	Tetracycline	30 µg	TE
Erythromycin	15 µg	E	Ticarcillin	75 µg	TIC
Fusidic Acid	10 µg	FA	Tobramycin	10 µg	TM
Imipenem	10 µg	IPM	Trimethoprim-Sulfamethoxazole [co-trimoxazole]	1.25 µg + 23.75 µg	SXT
Vancomycin	30 µg	VA			

RESULTS

Isolation and Identification of Lactic Acid Bacteria: A total of 76 isolates from the tested four samples could be identified and were divided into four genera: *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Enterococcus*. Thirty five isolates belonged to the genus *Lactobacillus*, 8 isolates to *Leuconostoc* and 13 isolates to *Lactococcus*. Members of the genus *Lactobacillus* dominated in all Dhan samples. Figure 1 illustrates the percentage of distribution of the 76 bacteria identified from traditional butter. All the lactococci isolates (a total of 13 isolates) belonged to *Lactococcus lactis* subsp. *cremoris.*

Four isolates from a total of 8 *Leuconostoc* isolates produced dextran from sucrose.

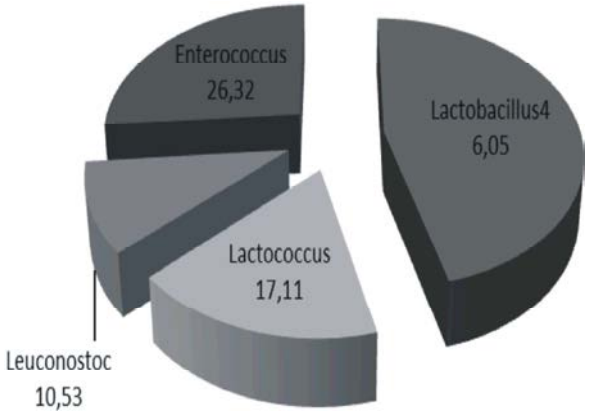

Fig. 1: Identity of the 76 bacterial strains isolated from indigenous traditional butter (Dhan)

Four of the *Leuconostoc* isolates were non-dextran-producing isolates. Three of them belonged to *Leuconostoc mesenteroids* subsp. *mesenteroides* species and one to *Leuconostoc lactis*. 76.92% of *Lactobacillus* isolates belonged to *Lactobacillus plantarum* species. *Lactobacillus delbrueckii* represented 84.61% of the total of lactobacilli isolates. Three isolates were *Lactobacillus delbrueckii* subsp. *Lactis,* 4 were *Lactobacillus delbrueckii subsp. delbrueckii* and 4 isolates belonged to *bulgaricus* subspecies. Five isolates were belonging to *Lactobacillus casei.* Eight isolates belonged to *Lactobacillus amylophilus* and finally one isolate belonged to *Lactobacillus brevis* (Table 2 and 3).

Proteolytic and Lipolytic Activity of Isolates: From all tested isolates, only two isolates were lipolytic B11 (*Lactobacillus delbrueckii* subsp. *Delbrueckii*) and A20 (*Lactobacillus delbrueckii* subsp. *Bulgaricus*) (Figure 4). For proteolytic activity, the use 1% of skimmed milk in PCA medium allowed to detect slight proteolytic activity. Three isolates belonging to *Lactococcus lactis* subsp. *cremoris* gave slight reaction with 1% and no activity was observed with 2% skimmed milk of added to PCA medium. The isolate G4 (*Lactobacillus plantarum*) had no proteolytic activity. All results of proteolytic activity were listed in Table 4.

Antimicrobial Activity: From candidate isolates belonging to different species, some isolates gave positive inhibition toward pathogenic bacteria. The supernatant of Lactococcus lactis subsp. Cremoris,

Table 2: Physiological and biochemical characteristics of lactobacillus strains isolated from Dhan

	1	2	3	4	5	6	7	8
Strains	G5, d4, S4	N4, H15, S1, N4	A24, d1, G1	H1, H11, H13, N3	S14, B5, N1, N11, d12, d14, H4, A2	S3, d3	s11	G4, H3, S12, N2 G2, A25, d11, S11 B3, B8
Gram	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-
CO_2 from Glucose	-	-	+	-	-	-	+	-
A.D.H	-	-	-	-	-	-	-	-
Temperature								
15°C	-	-	+	-	+	+	+	+
45°C	+	+	-	+	-	+	-	-
Sugar profile								
Xylose	-	-	-	-	-	-	-	-
Maltose	+	+	-	-	+	+	+	+
Galactose	-	-	+	-	+	+	-	+
D-sorbitol	-	-	-	-	-	+	-	+
Arabinose	-	-	-	-	-	-	+	-
Mannitol	-	-	+	-	-	+	-	+
L-rhamnose	-	-	-	-	-	+	-	-
Ducrose	-	+	-	-	+	-	-	+
Lactose	+	+	-	+	-	+	+	+
D-fructose	-	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+
Esculine	+	-	+	-	+	+	-	+

1:Lactobacillus delbrueckii subsp. Lactis; 2:Lactobacillus delbrueckii subsp. delbrueckii; 3:Lactobacillus casei subsp. casei; 4:Lactobacillus delbrueckii subsp. bulgaricus; 5:Lactobacillus amylophilus; 6:Lactobacillus casei subsp. rhamnosus; 7:Lactobacillus brevis; 8:Lactobacillus plantarum

Lactobacillus plantarum and Lactobacillus delbrueckii subsp. delbrueckii inhibit growth of *Escherichia coli, Staphylococcus aureus* and *Listeria innocua* respectively.

Antibiogram of Isolates: The behaviour of each isolate to different antibiotics in terms of sensitivity and resistance has been shown in Table 3. All isolates were found sensitive to most of the broad-spectrum antibiotics (Table 5).

DISCUSSION

Lactic acid bacteria predominate the total microflora; Lactobacillus dominates the totality of these flora. This dominance is initialized by the pre-acidification of milk prior to its transformation to butter. According to many studies, *Lactobacillus* dominates all traditional fermented dairy products [24-26]. *Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Lactobacillus delbrueckii subsp lactis, Leuconostoc lactis* and *Leuconostoc citreum* were identified in South African traditional fermented milks [24]. In this study, Lactobacillus was dominant flora with 23% from total lactic acid bacteria. Missotten *et al*. [25] found that 131 strains isolated from fermented liquid pig feed, out of 145 lactic acid bacteria are *Lactobacillus*. Khedid *et al.* [26] found that *Lactobacillus* isolated from camel milk represents 37.5% of LAB flora. This work also showed that the initial flora of raw milk contributes to that residual in the finished product, the method of traditional transformation and no pasteurization is carried out in these methods. The lactobacilli accounts represented 54.4% of the lactic flora in traditional fermented product containing manioc [27]. The most dominant species for this group is *Lactobacillus plantarum*. This species is largely known for its predominance for the *Lactobacillus* in almost the whole of the traditional products. This predominance is with a great number of properties, the acidification and the antimicrobial metabolites production will be quoted. *Lactobacillus delbruekii* also largely represented among the isolated strains. *Lactobacillus delbruekii* presented three subspecies; *lactis* (3 strains), delbruekii with 4 strains and finally *bulgaricus* with 5 strains. *Lactobacillus casei* was represented by 8 strains (*casei* and *rhamnosus*). The presence of the lactobacilli in this traditional product is largely due to the methodology used which consists in acidifying milk for 24 hours period before starting the separation of the fat contents. Milk thus acidified contributes to the selection of the acidifying flora. The following stage, separation of the fat contents, is made in an enclosure cook with anaerobic conditions, facilitating the development of the lactic CO_2 bacteria producing as *Leuconostoc* [28] which were present under species *Leuconostoc mesenteroides* subsp. *dextranicum*, strains producing dextran and *Leuconostoc mesenteroides* subsp. *mesenteroides*. The lactococcus ones were represented by only one species which was *Lactococcus lactis* subsp. *cremoris*. It will be announced that the phenotypic classification of this species gives

Table 3: Physiological and biochemical characteristics of coccus strains isolated from Dhan

	1	2	3	4
Souches	H10	B10, B11, M2, M11	M3, M5, M13	G6,G8,G10,G13 A7,A9,A20,H9,d9,d7,N9,d17,d18
Gram	+	+	+	+
Catalase	-	-	-	-
CO2 à partir du Glucose	+	+	+	-
A.D.H	-	-	-	-
Température				
15°C			+	+
45°C	-	-	-	-
Sugar pro?le				
xylose	-	-	+	+
Maltose	+	-	+	-
Galactose	+	-	+	+
D-sorbitol	-	-	-	-
Arabinose	-	-	-	-
Mannitol	-	+	-	-
L-rhamnose	-	-	-	-
Sucrose	-	-	+	-
Lactose	+	+	+	+
D-fructose	+	+	+	+
Glucose	+	+	+	+
Esculine	-	+	+	-

1:Leuconostoc lactis; 2:Leuconostoc mesenteroides subsp. dextranicum; 3:Leuconostoc mesenteroides subsp. mesenteroides; 4: Lactococcus lactis subsp. cremoris

Table 4: Proteolytic activity of isolated lactic acid bacteria

	Milieu PCA+Lait			Milieu PCA+Lait			Milieu PCA+Lait	
Isolate	1%	2%	Isolate	1%	2%	Isolate	1%	2%
d17	+	+	H6	Nd	-	A9	+	+
N11	+	+	d9	+	+	B13	+	+
N15	+	+	N13	-	-	A25	+	+
N2	+	+	N16	-	+	M2	+	+
S'2	+	+	S15	+	+	A8	+	-
S10	+	+	G8	+	+	M13	+	+
N9	+	+	B7	+	+	B7	+	-
S8	+	+	S17	+	+	M11	+	+
d18	+	+	S14	+	+	M5	+	+
S7	+	+	B5	Nd	+	G16	+	+
N14	+	+	A24	Nd	+	B4	+	+
S18	+	+	M6	-	+	H5	-	+
N7	+	+	G1	+	-	B11	+	+
N5	-	+	G6	+	-	d14	+	+
N12	+	+	B10	+	-	H5	+	+
S11	+	+	G4	-	-	d5	+	+
S12	+	+	A7	+	+	S13	+	+
d10	+	+	A19	+	+	N10	+	+
M7	+	+	G5	+	+	A21	+	+
G13	+	-	G12	+	+	A18	+	+
B8	+	+	B15	+	+	d12	+	+
d18	+	-	M12	-	+	H9	Nd	Nd
G2	+	+	M1	+	-	B3	+	+
M10	-	+	A27	+	+	A5	+	+
S6	+	-	S9	Nd	-	A20	+	+
d4	+	-	G15	+	+	G10	+	+

Nd: Not determined

Table 5: Antibiogram of representative isolates of lactic acid bacteria.

	Isolates					
Antibiotics	N11	M5	A20	d18	d14	B11
OFX	S^+	S^+	S^+	S^+	S^+	R
AN	S^+	S^+	S^+	S^+	S^+	S^+
NET	S^+	S^+	S^+	S^+	S^+	S^+
CF	S^+	S^+	S^+	S^+	S^+	S^+
CIP	S^+	S^+	S^+	S^+	S^+	R
TIC	S^+	S^+	S^+	S^+	S^+	S^+
OX1	R	R	S^+	S^+	R	R
CS	S^+	S^+	S^+	S^+	S^+	R
AMC	S^+	S^+	S^+	S^+	S^+	S^+
TM	S^+	S^+	S^+	S^+	S^+	S^+
CTX	S^+	S^+	S^+	S^+	S^+	S^+
FT	S^+	S^+	S^+	S^+	S^+	S^+
CAZ	R	S^+	S^+	S^+	S^+	S^+
PIP	S^+	Nd	S^+	S^+	S^+	S^+
FOX	S^+	S^+	S^+	S^+	S^+	S^+
TE	S^+	S^+	S^+	S^+	S^+	S^+
VA_{30}	R	R	S^+	R	S^+	R
L	S^+	S^+	S^+	S^+	S^+	S^+
RA	S^+	S^+	S^+	S^+	S^+	S^+

Nd: Not determined

OFX: Ofloxacin, AN: Amikacin, NET: Netilmicin, CF: Cephalothin, TIC: Ticarcillin, OX1: Oxacillin, CS: Colistin, AMC: Amoxycillin + Clavulanic Acid, TM: Tobramycin, CTX: Cefotaxime, FT: Nitrofurantoin, CAZ: Ceftazidime, PIP: Piperacillin, FOX: Cefoxitin, TE: Tetracycline, VA30: Vancomycin, L: Lincomycin, RA: Rifampin

ambiguous results. It was confirmed by several researches that the strains which were identified like *cremoris*, genotypically is lactis and screw-poured [29-31]. Generally, the species identified in the present study, were in good agreement with other studies [29-31].

In the concurrent work, the isolated lactic acid strains showed their capability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* by producing antimicrobial substance in the culture medium. This production was confirmed by using culture supernatant tested by well method diffusion. These two undesirable strains are known contaminants of the acidified dairy products.

The antibiotic resistance pattern of the lactic acid bacteria showed resistance to oxacilin and the vancomycin. Essid *et al.* [32] working on lactobacilli, found that almost the all strains are sensitive to the amikacin (30 µg), cefuroxime (30 µg), gentamicin (10 µg), nor?oxacine (10 µg) and streptomycin (10 µg). The same authors found 88.2 and 82.3% of the strains are resistant to erythromycin and rifampicin respectively. 70.5% of these strains were resistant to the ampicillin and the penicillin G. Nguyen *et al.* [33] found that *Lactobacillus plantarum* is resistant to tetracycline, gentamicin and penicillin and sensitive to erythromycin and ampicillin. Gevers *et al.* [34] reported that 100, 79 and 64% of the lactic bacteria isolated from fermented and dried sausage are resistant to tetracycline, gentamicin and penicillin respectively. It should be known that before using a lactic starter, it is important to check that the bacterial strains cannot transfer resistant genes [23]. The proteolytic and lipolytic activities are largely required for the selection of the lactic starters for the industry of several dairy products and especially that of cheeses as brings back several authors [32, 35]. The lactobacilli isolated from traditional butter did not express lipolytic activity. Two strains of *Leuconostoc* represented by the strain B and M5 expressed lipolytic activity. With these two strains *Lactococcus lactis* subsp *cremoris* A20 expressed same activity. Studies made by Ammor *et al.* [23] confirm our results by finding the majority of their strains of *Lactobacillus* unable to express lipolytic activity. In the same way other works arrive at the same conclusion [36, 37].

The most dominant flora in the examined Dhan samples was represented by the lactobacilli; *Lactobacillus plantarum* the most dominant species 28.57. The isolated lactic acid bacteria inhibit *Staphylococcus aureus* and *Escherichia coli*. These two undesirable strains are known contaminants of the acidified dairy products. Two strains of *Leuconostoc* and *Lactococcus lactis* subsp. *cremoris* A20 expressed lipolytic activity. The antibiotic susceptibility patterns of our strains showed almost total sensitivity to the used antibiotics.

REFERENCES

1. Leroy, F. and L. De Vuyst, 2004. Functional lactic acid bacteria starter cultures for the food fermentation industry. Trends in Food Science and Technology, 15: 67-78.
2. Caplice, E. and G.F. Fitzgerald, 1999. Food fermentation: Role of microorganisms in food production and preservation. Int. J. Food Microbiol., 50: 131-149.
3. Abdelbasset, M. and K. Djamila, 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk “Raïb”. African J. Biotechnol., 7: 2908-2914.
4. Cocolin, L., R. Foschino, G. Comi and M.G. Fortina, 2007. Description of the bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk. Food Microbiol., 24: 752-758.

5. Diop, M.B., R. Dibois-Dauphin, E. Tine, A.N. Jacqueline and P. Thonart, 2007. Bacteriocin producers from traditional food products. Biotechnologie, Agronomie, Société et Environnement, 11: 275-281.
6. Lasagno, M., V. Beoletto, F. Sesma, R. Raya, G. Font De Valdez and A. Eraso, 2002. Selection of bacteriocin producer strains of lactic acid bacteria from a dairy environment. Microbiologia, 25: 37-44.
7. Ravytz, F., S. Barbuti, M.A. Frustoli, G. Parolari, G. Saccani and L. De Vuyst, 2008. Competitiveness and antibacterial potential of bacteriocin-producing starter cultures in different types of fermented sausages. J. Food Protection, 71: 1817-1827.
8. Simonová, M., V. Strompfová, M. Marcináková, A. Lauková, S. Vesterlund, M.L.Moratalla, S. Bover-Cid and C. Vidal-Carou,2006. Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak Meat Products. Meat Sci., 73(4): 559-564.
9. De Man, J.C., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-135.
10. Mayeux, J.V., W.W.E. Sandine and P.R. Elliker, 1962. A selective medium for detecting *Leuconostoc* organisms in mixed strain starter cultures. J. Dairy Sci., 45: 655-656.
11. Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for lactic streptococci and their bacterio-phages. Applied Microbiol., 29: 807-8 13.
12. Guessas, B. and M. Kihal, 2004. Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats milk. Afr. J. Biotechnol., 3: 339-342.
13. Thomas, T.D., 1973. Agar medium for differentiation of *Streptococcus cremoris* from the other bacteria. NZ J. Dairy Sci. Technol., 8: 70-71.
14. Thomas, T.D. and G.C. Pritchard, 1987. Proteolytic enzymes of Dairy starter cultures. FEMS Microbiol. Rev., 46: 245-268.
15. Kempler, G.M. and L.L. McKay, 1980. Improved medium for detection of citrate-fermenting *Streptococcus lactis* subsp *diacetylactis*. J. Appl. Environ. Microbiol., 39: 956-927.
16. Samelis, J., F. Maurogenakis and J. Metaxopoulos, 1994. Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. Int. J. Food Microbiol., 23: 179-196.
17. Guessas, B., 2007. Les potentialités métaboliques des bactéries lactiques isolées du lait cru de chèvre dans le bio-contrôle de Staphylococcus aureus. Thèse de doctorat d'état. Département de biologie, Faculté des sciences, Université d'Oran, Es-Senia, pp: 165.
18. Sierra, G., 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substances. Antonie van Leeuwenhoek J. Microbiol. Serol., 23: 15-22.
19. Guiraud, J. and P. Galzy, 1980. L'analyse microbiologique dans les industries agroalimentaires. Collection Génie alimentaire, édition de l'usine nouvelle, Paris, pp: 239.
20. Gopinath, S.C.B., P. Anbu and A. Hilda, 2005. Extracellular enzymatic activity in fungi isolated from oil rich environments. The Mycological Society of japan and springer verlag Tokyo. Mycoscience, 46: 119-126.
21. Moulay, M., H. Aggad, Z. Benmechernene, B. Guessas, D.E. Henni and M. Kihal, 2006. Proteolytic activity of cultivable lactic acid bacteria isolated from Algerian raw goat's milk. World J. Dairy Food Sci., 1: 12-18.
22. Schillinger, U. and F.K. Lücke, 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. and Environ. Microbiol., 59: 1901-1906.
23. Ammor, S., G. Tauveron, E. Dufour and I. Chevallier, 2006. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: 1 - Screening and characterization of the antibacterial compounds. Food Control, 17: 454-461.
24. Beukes, E.M., B.H. Bester and J.F. Mostert, 2001. The microbiology of South African traditional fermented milks. Int. J. Food Microbiol., 63: 189-197.
25. Missotten, J.A.M., J. Goris, J. Michiels, E. Van Coillie, L. Herman, S. De Smet, N.A. Dierick and M. Heyndrickx, 2009. Screening of isolated lactic acid bacteria as potential beneficial strains for fermented liquid pig feed production. Animal Feed Science and Technol., 150: 122-138.
26. Khedid, K., M. Faid, A. Mokhtari, A. Soulaymani and A. Zinedine, 2009. Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. Microbiol. Res., 164: 81-91.

27. Kostinek, M., I. Specht, V.A. Edward, C. Pinto and M. Egounlety, 2007. Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. Int. J. Food Microbiol., 114: 342-351.
28. Kihal, M., H. Prevost, D.E. Henni, Z. Benmechernene and C. Diviès, 2007. Carbon Dioxide Production by Leuconostoc mesenteroides Grown in Single and Mixed Culture with Lactococcus lactis in Skimmed Milk. World Journal of Dairy and Food Sci., 2: 62-68.
29. Godon, J.J., C. Delorme, S.D. Ehrlich and P. Renault, 1992. Divergence of genomic sequences between *Lactococcus lactis* subsp. *lactis* and *lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol., 58: 4045-4047.
30. Salama, S.M., W.E. Sandine and J.S. Goviannoni, 1993. Isolation of *Lactococcus lactis* subsp. *cremoris* from nature by colony hybridization with rRNA probes. Appl. Environ. Microbiol., 59: 3941-3945.
31. Charteris, W.P., P.M. Kelly, L. Morelli and J.K. Collins, 2001. Quality control *Lactobacillus* isolates for use with the API50 CH and API ZYM systems at 37 1C. J. Basic Microbiol., 41: 241-251.
32. Essid, I., M. Medin and M. Hassouna, 2009. Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. Meat Sci., 81: 203-208.
33. Nguyen, T.D., J.H. Kang and M.S. Lee, 2007. Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterollowering effects. Int. J. Food Microbiol., 113: 358-61.
34. Gevers, D., M. Danielsen, G. Huys and J. Swings, 2003. Molecular characterization of tet(M) genes in Lactobacillus isolates from different types of fermented dry sausage. Applied and Environ. Microbiol., 69: 1270-1275.
35. Nieto-Arribas, P., S. Seseña, J.M. Poveda, L. Palop and L. Cabezas, 2010. Genotypic and technological characterization of *Leuconostoc* isolates to be used as adjunct starters in Manchego cheese manufacture. Food Microbiol., 27: 85-93.
36. Kenneally, P.M., R.G. Leuschner and E.K. Arendt, 1998. Evaluation of the lipolytic activity of starter cultures for meat fermentation purposes. J. Appl. Microbiol., 84: 839-846.
37. Papamaloni, E., N. Tzanetakis, E. Litopoulou-Tzanetaki and P. Kotzekidou, 2003. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. Meat Sci., 65: 859-867.