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Identification and Characterization of Lactic Acid Bacteria Isolated from Rural Traditional Cheese (Jben) of Djelfa Province

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Abstract: Five samples of traditional cheese (Jben) collected in rural area of Djelfa, Algeria. Were studied by determination of morphological, cultural, physiological and biochemical characteristics. Among 104 isolates 29 lactic acid bacterial (LAB) strains were isolated and purified. This study shows that the biodiversity of this traditional cheese studied is characterized by enterococci, lactobacilli and lactococci. The most common lactic acid bacteria belonging to the species *Lactococcus lactis* (10.34%), *Lactococcus raffinolactis* (6.90%), *Lactococcus cremoris* (06.90%), *Lactobacillus Plantarium* (13.79%), *Lactobacillus rhamnosus* (10.34%), *Lactobacillus fermentum* (10.34%), *Lactobacillus acidophilus* (13.79%), *Lactobacillus casei* (10.34%), *Lactobacillus helveticus* (06.90%) and other species of the genus *Enterococcus* (10.34%). The samples pH average was 4, 42 ± 0.15, mean titratable acidity value was 79.4 ± 3, 11 °D and microbiological analysis results were; total mesophilic aerobic bacteria 6.12 x 10^6 UFC/g, Coliform bacteria 1.04 x 10^4 UFC/g, Yeast 1.08 x 10^4 UFC/g. *Staphylococcus, Salmonella* and Moulds weren't detected. In this work, we tested the acidifying power, proteolytic activity and the antimicrobial effect of these LAB isolated against *Bacillus subtilis, Staphylococcus aureus and Escherichia coli*. The strains (GM13, GM11, GM33, GM20, GM60 and GM40) were selected for its strong bactericidal activity against *Staphylococcus aureus*. The action of the bacteriocins was eliminated by proteolytic enzymes.

Key words: Bacteriocins · Jben · Lactic acid bacteria · Microbiological analysis · Proteolytic activity

INTRODUCTION

The original production of fermented milk products derived from the need to prolong the shelf life of milk instead of being disposed. Yogurt manufacture was initially based on knowledge and empirical processes without standard procedures or investigation of the steps that occur during the entire process. Only after the late 20th century, when yogurt became a profitable commercial good, its manufacture became industrialized and the processes were standardized. During the last 20 years, interest in yogurt manufacture has increased tremendously for scientific and commercial reasons. Scientific findings have suggested new dairy products that benefit human health (probiotic cultures, fortification with bioactive compounds) as well as with improved sensory, especially textural characteristics. Thus, consumer demand for yogurt and similar fermented dairy products has increased [1]. These which product is based on the metabolic activity of lactic acid bacteria (LAB) ferment sugars, especially glucose and galactose, so to produce lactic acid and aroma substances that give typical flavors and tastes to fermented products. Several types of fermented milk products have been reported to

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exist throughout the world [2]. The most popular of them in Algeria are Jben, Lben, Klila, Raúb and Bouhazza. In a variety of ecological niches, micro-organisms compete with each other for survival and through evolution from unique flora. The traditional soft white cheese (Jben) made from non-pasteurized milk, is characterized by a total dry matter of about 35% and a pH lower than 4.5. Nowadays, Jben is also prepared from pasteurized milk. The final characteristics of a typical Jben are variable and affected by preparation of the cheese. Its production does not conform to official hygiene and other regulatory standards and follows informal marketing routes. The microbiota of traditional cheese (Jben) is dominated LAB present in a range of at least 10^8–10^9 UFC/g [3]. The LAB have played along and important role in Food technology and have a long history of use by man for food production and food preservation. These organisms are able to produce antimicrobial compounds against competing flora, including food-borne spoilage and pathogenic bacteria. The LAB comprise a clade of Gram positive, acid tolerant, non-sporulation, non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics. These bacteria are usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end product of carbohydrate fermentation. This trait has historically linked LAB with food fermentation as acidification inhibits the growth of spoilage agents. The LAB group comprises the genera *Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Aerococcus, Alloicoccus, Dolosigranulum, Enterococcus, Globicatella, Lactospaera, Oenococcus, Carnobacterium, Tetragenococcus, Vagococcus* and *Weissella*. Historically, the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* form the core of the LAB group [4]. Lactic acid bacteria have a long history of safe use in the production of fermented foods and beverages. Whereas natural, spontaneous fermentation processes are still common practice in many cases, there is a clear trend towards the use of multiple, defined starter cultures to have a controlled industrial fermentation process. The current role and industrial relevance of the natural, wild type strains which do not belong to the starter culture but originate from the raw material or the production environment is regarded with interest [5]. The lactic acid fermentation, which these bacteria carry out, has long been known and applied by humans for making different foodstuffs. In addition, they strongly determine the flavor, texture and, frequently, the nutritional value of food. They should possess stable fermentation characteristics and should be resistant to bacteriophages [6]. Proteolytic system is considered one of the most important biochemical processes involved in manufacturing many fermented dairy products, for example in cheese manufacture, the proteolysis of casein is thought to play a pivotal role because amino acids resulting from proteolysis are the major precursors of specific flavor compounds, such as various alcohols, aldehydes, acids, esters and sulfur compounds [7]. Bacteriocins usually have low molecular weight (rarely over 10 kDa) they undergo posttranslational modification and can be easily degraded by proteolytic enzymes especially by the proteases of the mammalian gastrointestinal tract, which makes them safe for human consumption. Bacteriocins are in general cationic, amphipathic molecules as they contain an excess of lysyl and arginyl residues. They are usually unstructured when they are incorporated in aqueous solutions but when exposed to structure promoting solvents such as triofluroethanol or mixed with anionic phospholipids membranes they form a helical structure. Among the Gram positive (+) bacteria, the LAB have gained particular attention nowaday, due to the production of bacteriocins. These substances can be applied in the food industry as natural preservatives [8]. The purposes of this study firstly were the isolation and taxonomic determination of large number lactic acid bacteria from traditional cheese and characterization of different groups of microflora, acidifying power, proteolytic activity and an antimicrobial using classical methods, with a microbiological study to highlight the hygienic quality of these products.

MATERIALS AND METHODS

Rural Area Study: The wilaya of Djelfa is located in the central part of northern Algeria (Figure 1). This centrality allows the wilaya to develop more and more. It represents the perfect link from North to South of the country and from east to west and an undisputed point of passage. Due to the conditions of its natural environment and the extent of its territory, the Wilaya of Djelfa is a steppic Wilaya where sheep farming predominates, its main vocation is pastoral [9].

Samples Collection: Five samples studied traditional Cheese (Jben) were collected from the rural area of Djelfa province, Algeria. They were directly transferred by refrigerated (4°C) containers to the laboratory and analyzed immediately without further storage. A standard procedure to measure the pH of cheese (BS 770-5:1976)

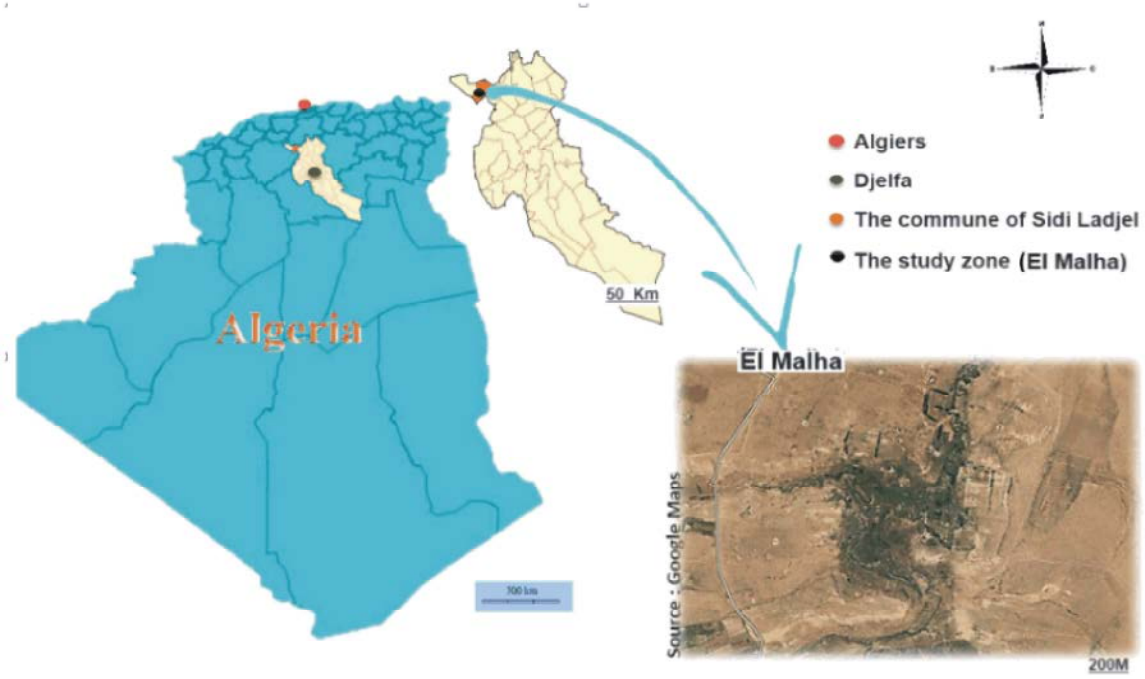

Fig. 1: Location of the sampling area

[10] was used. Briefly, a slurry was created from a cheese sample using distilled water at 20°C and the pH of the cheese suspension was recorded with a standard laboratory electrode-based pH meter with an Orion Research type combination electrode and previously calibrated with buffer solutions at pH 4 and pH 7. All samples were measured in duplicate and the mean values are provided [11]. Transfer 10 ml of sample to a small beaker and Add 5 drops of phenolphthalein to 1% indicator. Titrate the sample with 0.1 N NaOH. Note that the sample should be just barely pink [12].

Microbiological Analysis: Traditional microbiological methods and media were used for the isolation and enumeration of Total Aerobic Mesophilic Bacteria (TAMB), there have been made decimal dilutions in physiological water. From each dilution it was distributed, with sterile dropper, 1ml in each of the three Petri plates in each Petri plates was mold agar melted and cooled at 40 – 45°C, it was stirred and incubated for 24 hours at 37°C. It was assessed the mean of cfu /g product [13]. In addition, there has been used the method of establishing coliform bacteria number by colonies counting (ISO 4832). From the decimal dilutions it is distributed 1 ml in two Petri plates. In each plate is mold VRB agar (agar and lactose, bile salts, crystal violet and indicator), melted and cooled at 45°C. The above prepared plates are incubated at 35°C for 24 hours. After the incubation the red-purple colonies are counted and the mean value is calculated with the formula: $N = \Sigma c / (n1 + 0.1 n2) d$ (where ΣC is the sum of counted colonies; n1 is the number of plates to be counted from the first dilution; n2 is the number of plates to be counted from the second dilution; d is the dilution rate corresponding to the first used dilution) [13]. In order to establish the pathogen and opportunistic germs species as: *Staphylococcus* and Coagulase Positive *Staphylococcus* (CPS) were enumerated in Baird-Parker Agar (BD, Becton Dickinson and Company, France) supplemented with egg yolk and tellurite. The plates were incubated at 37°C for 24-48 h. After growth, suspicious colonies were counted. The colonies were classified as typical for S. aureus (jet black to dark gray, smooth, convex, entire margins with an opaque zone and a clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies, entire margin without a halo) [14]. For the detection of *Salmonella*, twenty five grams of cheese were suspended in 225 ml of buffered peptone water and incubated for 24 h at 37 °C for pre enrichment. One milliliter of pre enrichment broth was transferred to 10 ml of tetrathionate broth (Selective Enrichment) and incubated for 24 h at 42°C. One loop-full of tetrathionate broth was streaked onto *Salmonella* and *Shigella* agar (SSA) and Brilliants green agar (Selective media) and incubated at 37°C for 24 h incubation [15].

For establishing the Yeasts number and the Molds number, 10 g was aseptically removed homogenized in 50 ml of sterile 1% peptone water for 5 minutes at normal speed [16]. Samples were serially diluted and plated on PDA (potato dextrose agar). After the agar is gelling, put the flat plate upside down and incubate 5 days at 28°C ± 1°C. Observe and take record. Visualizing or use magnifier if necessary, take record of the yeasts and molds corresponding to the different dilution ratio. Expressed as CFU (colony forming units). Select the plate with counts between 10 CFU – 150 CFU, count the yeasts and molds respectively according to their appearance (GB 9678.2-2014) [17]. LAB counting were performed on MRS agar at pH 6.2 incubated at 30°C for 48h.

Study of Lactic Microbiota: Enumeration of LAB was determined using various elective media, MRS agar [18], 17 agar [19] agar and MSE [20].After incubation, colonies were enumerated, recorded as colony forming units (cfu) per liter of milk. The colonies were randomly picked from plates with 30–300 colonies and transferred in 10 ml of appropriate broth. The selected colonies were purified by repeated streaking on the appropriate agar media. LAB strains were kept on MRS agar slant at 4 °C and streaked every 4 weeks. Prior to use, LAB strains were activated in MRS broth at 30 °C for 24 h and subcultured in MRS agar at 30 °C for 24 h [21]. The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics. The used tests were: Gram reaction; production of catalase, cytochromes oxidase and hydrogen peroxide; growth at different temperatures15, 30, 37 and 45 °C; growth at different pH values; growth at different NaCl concentrations [22]. Hydrolysis of arginine was tested in M16BPC [23]. Growth in the presence of 4 and 6.5% NaCl performed in MRS broth for 5 days. Utilization of citrate was realized in Kempler and McKay medium [24]. Production of acetone from glucose was determined using Voges-Proskauer test [25].Gas production from glucose was assessed by inoculation of cultures into 5 mL MRS broth containing inverted Durham tubes and incubating at 35°C for 2 days. All strains were tested for fermentation capacity of different carbohydrates was determined on MRS-BCP broth medium, by using the carbon source, which was added to the sterile basal medium as filter sterilized solution to a final concentration of 1%. Carbohydrates utilization was evaluated at the 24 and 48h. The following 15 sugars were used for fermentation all of the isolates: Lactose, Xylose, arabinose, cellobiose, esculin, maltose, mannose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose and trehalose. For preparing anaerobic conditions, two drops of sterile liquid paraffine were settled in each tube after inoculation [26].

Technological Study: The technological ability of the studied strains was evaluated according to the acidification rate terms and coagulation of skim milk under our experimental conditions and the study of the proteolytic activity of our isolated strains. for the study of acidifying activity, the strains were initially grown in MRS broth and then in sterile reconstituted skim milk supplemented with yeast extract (0.3%) and glucose (0.2%) for two successive subcultures. Sterile reconstituted skim milk (100 ml) was inoculated with 1% of an 18h pre-culture [27]. After gentle agitation culture is divided into tube (10ml/tube) and incubated at 30 °C. At a regular interval time, samples were aseptically collected every 2 h. A volume of 1 ml culture samples was used for making suitable serial dilutions up to 10^{-8} by incorporating1ml into 9ml of sterile saline water in sterile tubes. Enumeration of LAB was deter- mined using selective media, MRS agar. The generation time and growth rate were calculated in the exponential growth phase. The determination of acidity during growth in skim milk is performed according to the method described by Accolas *et al*. [28] using NaOH (N/9) in the presence of phenolphthalein indicator (1% in alcohol). Samples were plated on MRS plates containing 1% (w/v) skimmed milk (pour plate method), incubated under anaerobic conditions at 37±1°C for 48h. The proteolytic LAB strains were identified by the presence of clear zone around the colonies. These colonies were picked up and further tested for their lipolytic activity by streaking them on to MRS plates containing 1% (v/v) of tributyrin as substrate [29]. Plates were incubated at 37°C for 4 days and observed daily for halo formation around the colonies. The radius of the halo formation (in mm) at the end of incubation was measured [30, 31]. As for the proteolytic activity residual proteins concentrations in the culture were estimated by a Coomassie G-250 binding procedure [32]. The isolates of LAB that exhibited proteolytic and characterized by different biochemical tests were further tested for their antibacterial activity against different pathogens. The many methods described for the detection of isolates bacteriocin-producing lactic acid is based on the premise that these protein substances can diffuse into a solid culture medium or semisolid which was previously inoculated with a target strains, *Bacillus subtilis* ATCC 93, *Staphylococcus aureus* ATCC 65 38 and *Escherichia coli* ATCC, L 25 922, were obtained from

the Educational Laboratory of the M'sila University. The bacteriocin production inhibitor is detected by the power of the filtrate microorganism tested growth target seed. Isolates of lactic acid bacteria after culture on medium MRS at pH 6.8, incubation at 30°C were tested for their anti-bacterial activity following diffusion method agar TSA (Tryptic Soy Agar, Difco, Detroit, USA) [33]. The supernatant containing the crude extract is collected by centrifugation bactériocinique adjusted to neutral pH of 6.5 to 7 with 10 M NaOH neutralizing the extract bactériocinique which eliminates the effect of organic acids. The extract was then filtered on millipore filters sterile 0.22 µ in diameter, the antimicrobial activity was determined for each selected isolate of LAB. Petri dishes were overlaid with 15 ml of molten agar (1%), inoculated with 30 µl of an overnight culture of the indicator microorganism, in which wells were formed. Wells, mm in diameter and of 30 µl in capacity, were formed by carving the agar with a cork borer. Afterwards, 30 µl of an overnight culture of the putative inhibitor strain were placed in each well. The plates were then incubated aerobically for 24 h at a temperature conducive for 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 (2 mm) of the inhibition zone displayed [34, 35].

Statistical Analysis: Average colony forming units of microbial load was calculated using descriptive statistics of spread sheet Microsoft excel.

RESULTS AND DISCUSSION

Physicochemical Analysis: The results of physicochemical analysis were shown in Table 1. Where pH range for traditional cheeses (Jben) was 3. 62 ± 0, 4 to 5, 02 ± 0, 01 with an average of 4. 42 ± 0.15 these values are similar to those found by Keyvani [36]. Titratable acidity of traditional cheese samples varies from values as low as 62. 33 ± 3, 06 °D to values as high as 91 ± 4 °D. Mean titratable acidity value was 79.4 ± 3. 11 °D, these values are almost similar to that reported by Mennane *et al.* [37].and Rhiat *et al*. [38].

Microbiological Analysis: Many of the common enteric pathogens such as *Salmonella*, *Escherichia coli O157: H7* and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats. Effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk [39], consumers' requirements for traditional fermented dairy food products are generally increased due to their proved gastronomic quality and positive effects on human health. However, the tightened legislation on food safety results in lower production flexibility, homogeneity in the food production and in the loss of food diversity and traditional specificity. Hence, the preparation of well-defined functional autochthonous starter cultures for production of traditional cheeses under controlled conditions, using the standardized traditional technology, is crucial [40]. The results of some microbiological properties of traditional cheese (Jben) are presented in Table 2. The Total Aerobic Mesophilic bacteria (TAMB) counts ranged from 1.01 x 10^6UFC/g and 1.4 x 10^7UFC/g with the average of 6.12 x 10^6UFC/g. The Coliform counts ranged from 0.2 x 10^4UFC/g and 2.3 x 10^4UFC/g with the average count of coliform bacteria was 1.04 x 10^4UFC/g and pathogenic bacteria *Staphylococcus* and *Salmonella* were not detected. The lactic acid bacteria are by the major microbial group in traditional cheese (Jben), the average number of LAB was 7.1 x 10^5UFC/g. The average Yeasts counts determined was from 1.08 x 10^4 UFC/g (Figure 2). According to the results obtained, in this research, the counts TAMB, Coliform bacteria and presence Yeasts in traditional cheese (Jben) were higher than the upper limits given in European Commission EC [41]. It is not surprising to obtain high microbiological counts in cheese with artisanal manufacturing methods due to the use of unpasteurized milk [42].

Isolation and Identification of Lactic Acid Bacteria: The enumeration of the lactic flora on MRS and M17 media gives respective mean values of 7.1 x 10^5 CFU/g and 2.28 x 10^6 CFU/g, respectively. It was found similarities with Traditional Koopeh Cheese, according to Hassanzadazar and Ehsani [44]. The characteristics of LAB are shown in Tables 3. All isolates were Gram-positive and catalase-negative bacteria. Twenty nine lactic acid bacterial (LAB) strains were isolated and purified. This study shows that the biodiversity of traditional cheese (Jben*)* studied is characterized by enterococci, lactobacilli and lactococci. The most common lactic acid bacteria belonging to the species *Lactococcus lactis* (Group 1: 10.34%), *Lactococcus raffinolactis*(Group 2: 6.90%), *Lactococcus cremoris* (Group 3: 06.90%), *Lactobacillus Plantarium* (Group 4: 13.79%), *Lactobacillus rhamnosus* (Group 5: 10.34%), *Lactobacillus fermentum* (Group 6: 10.34%),

Table 1: pH and acidity of traditional cheese samples

	pH					Acidity				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Mean	4, 133	4, 417	4, 900	3, 617	5, 020	76, 00	68, 67	65, 00	91, 00	62, 33
SD	0, 06110	0, 09713	0, 1153	0, 3837	0, 1054	1, 000	3, 512	4, 000	4, 000	3, 055
SE	0, 03528	0, 05608	0, 06658	0, 2215	0, 06083	0, 5774	2, 028	2, 309	2, 309	1, 764

SD: Standard deviation, SE: Standard error. S: Sample

Table 2: Results of microbiological analysis (UFC/g) of traditional cheeses (Jben)

Microbiological analysis	S1	S2	S3	S4	S5	M ± SD	Norm
Total Aerobic Mesophilic bacteria x 10^6	1.2	1.3	2.10	12	14	5.92± 6.53	10^5/g [41]
Coliform x 10^4	0.2	0.4	1.1	1.2	2.3	1.04± 0.83	10/g[41]
Yeasts x 10^4	0.3	0.4	1.2	1.4	2.1	1.08± 0.75	10^2/g [43]
Staphylococcus	Abs	Abs	Abs	Abs	Abs	Abs	0/1g[41]
Salmonella (1g)	Abs	Abs	Abs	Abs	Abs	Abs	0/1g[41]

S: Samples, M: Mean, SD: Standard deviation, Abs: Absence,

Table 3: Morphological, cultural, physiological and Biochemical Characteristics of the isolates

	Strains croups									
Characteristics	1	2	3	4	5	6	7	8	9	10
Number of isolates	3	3	2	4	3	3	4	3	2	3
Gas from glucose	+	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	-	-
Hydrolysis of:										
ADH	+	+	-	-	-	+	-	-	+	+
Citrate	+	-	+	-	-	+	-	+	-	-
Growth at different temperature (°C)										
15	-	+	+	+	+	-	-	+	-	+
30	+	+	+	+	+	+	+	+	+	+
45	+	-	-	-	+	+	+	-	+	-
Growth at different pH										
6.5	+	+	+	+	+	+	+	+	+	+
9.6	-	-	-	-	-	-	-	-	-	+
Growth in the presence of NaCl %										
4	+	-	-	+	+	-	+	-	+	+
6.5	-	-	-	-	-	-	-	-	-	+
9.6	-	-	-	-	-	-	-	-	-	+
Sugar Fermentation:										
Arabinose	-	-	-	-	-	+	-	-	+	+
Cellobiose	+	+	-	+	+	+	+	+	-	+
Mannitol	-	-	-	+	+	-	-	+	-	+
Mannose	+	-	+	+	+	+	+	+	-	+
Melibiose	-	+	-	+	+	+	-	-	-	+
Raffinose	-	+	-	+	-	+	-	-	-	+
Ribose	+	-	-	+	-	-	-	+	-	-
Lactose	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	+	+	-	-	-	-	+
Sorbitol	-	-	-	-	-	-	+	+	-	-
Xylose	-	-	-	-	-	+	-	-	+	+
Trehalose	+	+	-	+	+	-	-	+	-	-
Maltose	+	+	-	+	+	+	+	+	-	+
Esculin	+	-	-	+	+	-	-	+	-	+
Sucrose	-	-	-	+	+	+	+	-	-	+

(+): Positive reaction, (-): Negative reaction, Group 1: GM5, GM11 and GM88. Group 2: GM10 and GM22. Group 3: GM91 and GM15. Group 4: GM23, GM28, GM40 and GM80. Group 5: GM77, GM60 and GM45. Group 6: GM31, GM07 and GM2. Group 7: GM55, GM95, GM33 and GM19. Group 8: GM20, GM52 and GM12. Group 9: GM30 and GM71. Group 10: GM13, GM93 and GM66.

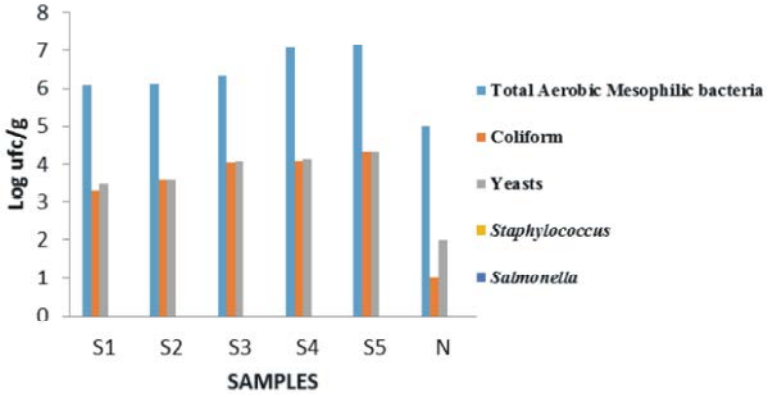

Fig. 2: Microbiological analysis (Log ufc/g) of traditional cheeses (Jben)

Lactobacillus acidophilus (Group 7: 13.79%), *Lactobacillus casei* (Group 8: 10.34%), *Lactobacillus helveticus* (Group 9: 06.90%) and other species of the genus *Enterococcus* (Group 10: 10.34%).We have divided the Lactobacilli group into three subgroups according to Orla-jensen [41], as follows: *Lactobacillus plantarum, Lactobacillus casei* and *Lactobacillus Johnsoni* are mesophilic facultative homo-fermentative, *Lactobacillus helveticus, Lactobacillus acidophilus* and *Lactobacillus fermentum* which are thermophilic obligate homo-fermentative [46]. Growth at 45°C, pH 9.6 or with 6.5% NaCl was tested for all spherical morphology isolates. Three isolates were not able to grow under these conditions but were able to growth at 10°C as described by Axelsson [47] for *Lactococcus* sp (*Lactococcus lactis, Lactococcus raffinolactis* and *Lactococcus* cremoris [48]. The rest of selected isolates were cocci occurring in pairs or short chains, facultative anaerobes and tolerant to a wide range of conditions: temperature (10 – 45°C), pH (4.5 - 10.0) and high sodium chloride concentrations, they tentatively referred to *Enterococcus* sp [41].

Technological Study: The variation of acidification was monitored for all strains, as shown in Figure 3, Figure 4 and Figure 5. The diminution of pH of the milk is due to the production of lactic acids from lactose fermentation [50]. The amount of lactic acid varies according the strains and their capacity and the rate of degradation of the lactose. Thus, according to their ability of acidification, the strains were divided as following: highly acidifying strains include GM55, GM19, GM05, GM11, GM33, GM65, GM45, GM13 and GM93, that coagulate milk before 18 hours of incubation, low acidifying strains GM88, GM22, GM91, GM23, GM80, GM02, GM95 and GM28, that coagulate milk after 18 hours of incubation and the remaining strains coagulate milk among 18 to 24 hours of incubation. The initial pH of skim milk was 6.2 to 6.5 for all the tested strains. Then, the pH decreases with time to reach 5.01 to 4.87 in highly proteolysis strains. Regarding the acidity we note that after 2 hours of incubation, the amount of lactic acid is measured (18-25°D) for all our strains. The acidity increases with the time of a variable way to arrive until 87 °D after 24 hours with strain GM19 and up of 35°D with the strain GM02.

The production of the lactic acid at summer followed according to time by using the pure cultures of selected stocks, this phenomenon (acidifying activity) is often used in dairy industry. The factor more used in the variation of the kinetics of acidification, is the aptitude of the strains had a proteolytic activity, which is coded by chromosomal extra equipment which is the plasmid [51]. The appearance of the zones of the proteolytic activity in the concentrations 1 and 2 % is very easily detected. While in concentrations 3 and 4 % detection is very low and fully absent in high concentration 5 %. In this case all the strains selected gave a zone of lyses on milieu PCA skimmed milk 1 and 2% with a variation of 01.7 to 07.5 mm. Thus they have a strong proteolytic activity (Table 4). One chooses the adequate concentration lower than 2 %, to obtain strains with a great proteolytic power. We have chosen to strongly proteolytic strains to the metering casein. from which it can be said that the amount of casein decreases rapidly with time in strains strongly proteolytic (Group 1, Group 4 and Group 7) with a mean velocity of consumption equalizes with (722µg/h). These similar results with the results obtained by Atanasovaa *et al*. [52]. For strains *Lactobacillus lactis.*

Antibacterial Activity: The antimicrobial activity of lactic acid bacteria isolated from traditional cheese (Jben) were detected using the method of well diffusion test on the basis of their ability to inhibit the growth of the indicator

Table 4: Hydrolysis zones of the proteolytic activity on PCA medium with the following concentration (1, 1.5, 2 and 3% of skimmed milk) at 30 °C for 24 hours of incubation

		Lactococcus sp			*Lactobacillus* sp						*Enterococcus* sp
		Mean of hydrolysis zone (mm)									
Strains group		1	2	3	4	5	6	7	8	9	10
Concentration of skimmed milk	1%	7.3	1.8	3.1	7.5	2.3	1.0	7.2	3.6	1.1	2.0
	2%	6.2	1.7	3.4	6.2	1.1	0.2	5.4	4.5	0.8	1.7
	3%	1.8	0.5	1.9	1.9	0.9	0.1	1.7	1.9	0.3	0.5
	4%	0.2	0.5	0.9	1.3	0.3	0.0	0.3	0.2	0.0	0.0
	5%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Group 1: GM5, GM11 and GM88. Group 2: GM10 and GM22. Group 3: GM91 and GM15. Group 4: GM23, GM28, GM40 and GM80. Group 5: GM77, GM60 and GM45. Group 6: GM31, GM07 and GM2. Group 7: GM55, GM95, GM33 and GM19. Group 8: GM20, GM52 and GM12. Group 9: GM30 and GM71. Group 10: GM13, GM93 and GM66

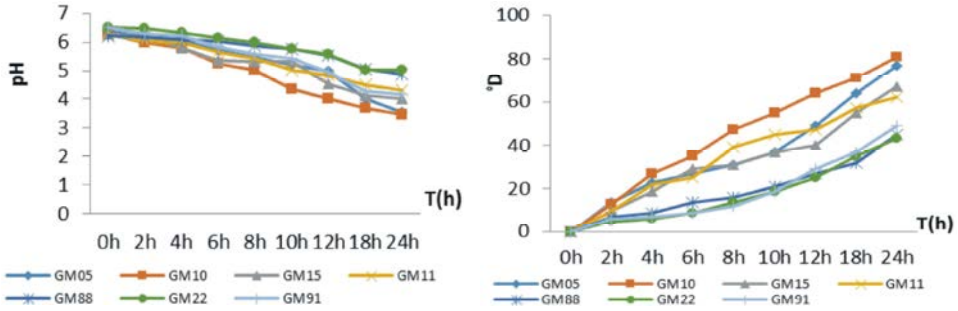

Fig. 3: Kinetics of pH and dornic acidity evolution of growth of the *Lactococcus* sp.

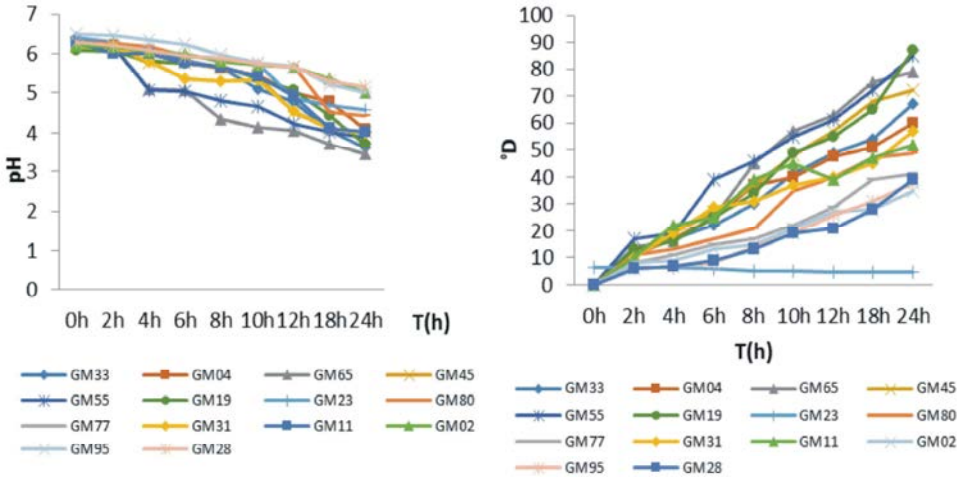

Fig. 4: Kinetics of pH and dornic acidity evolution of growth of the *Lactobacillus* sp.

isolate *S. aureus* ATCC 65 38 (Figure 6). Based on the results, a total of 5 different traditional cheese (Jben) samples analyzed. The strains of the group 01, group 07 and group 08 showed the largest zone of growth inhibition was selected for further strain developmental studies (Table 5).With regard to the results of other indicator bacteria *Bacillus subtilis* ATCC 93 and *Escherichia coli* ATCC, L 25 922.Were found reflect their ability to inhibition.

Inhibitor compounds produced by strains inhibitors showed different patterns of sensitivity. The strains (GM20, GM52, GM13, GM93, GM07, GM02, GM55, GM19, GM91, GM28, GM40, GM77, GM11, GM28, GM10 and GM22) were completely inactivated by α-chymotrypsin alone which was resistant to pepsin (GM95, GM30 and GM66), whereas the compounds produced by GM88, GM60, GM55, GM52 and GM93 isolates were inactivated after treatment with the lipase, indicating that these

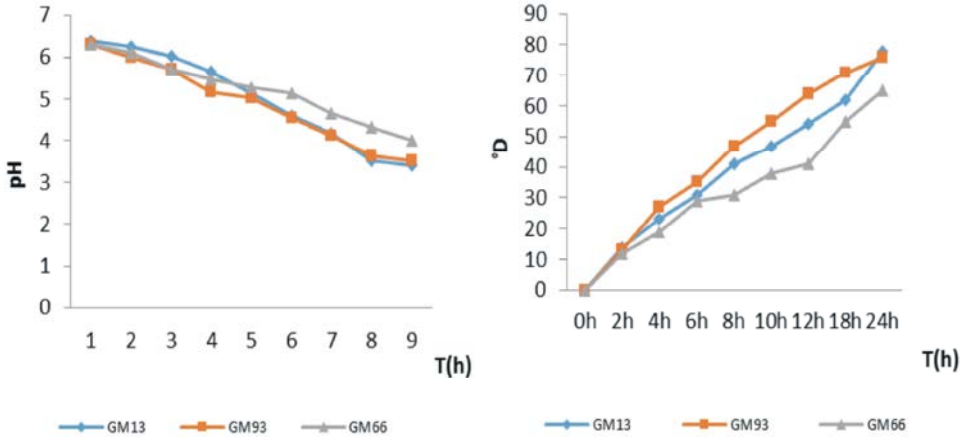

Fig. 5: Kinetics of pH and dornic acidity evolution of growth of the *Enterococcus* sp.

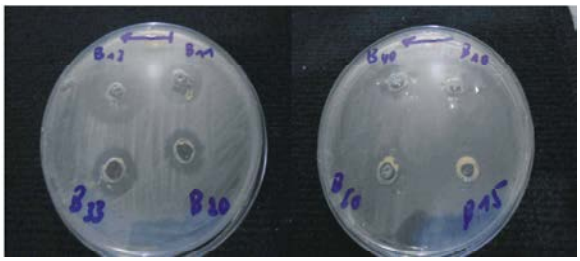

Fig. 6: Antibacterial activity of selected LAB from traditional cheese (Jben) against *Staphylococcus aureus* ATCC 65 38 by the cell-free supernatant of the producing isolates using the agar well-diffusion assay. B13: GM13, B11: GM11, B33: GM33, B20: GM20, B40: GM40, B10: GM10, B60: GM60 and B15: GM15.

Table 5: Screening of LAB for antibacterial activity against *S. aureus* ATCC 65 38

Strains group	Strains code	Diameter of inhibitory zone Agar well-diffusion method (mm)
Lactococcus lactis	GM05	+
	GM11	+++
	GM88	-
Lactococcus raffinolactis	GM10	+++
	GM22	+
Lactococcus cremoris	GM91	+
	GM15	+++
Lactobacillus Plantarium	GM23	+
	GM28	-
	GM40	+++
	GM80	-
Lactobacillus rhamnosus	GM77	+
	GM60	+++
	GM45	+
Lactobacillus fermentum	GM31	+
	GM07	+++
	GM02	-
Lactobacillus acidophilus	GM55	++
	GM95	++
	GM33	++
	GM19	+++
Lactobacillus casei	GM20	+++
	GM52	+
	GM12	-
Lactobacillus helveticus	GM30	+
	GM71	+
Enterococcus. sp	GM13	+++
	GM93	+
	GM66	-

(+): weak (3-6), (+): intermediate (7-11), (+): strong (12-15), (-): no growth.

Table 6: Action of proteolytic enzymes, lipase enzyme pH and heat treatment on the antimicrobial activity of crude extracts against the growth of *S. aureus* ATCC 65 38F

	Treatments							
	Enzymatic treatments			pH treatments			Heat treatments (°C/20min)	
Crude extracts	α-chymotrypsin	Pepsin	Lipase	3	5	7	80	120
GM05	+	+	+	-	-	+	+	-
GM11	-	-	+	-	+	+	+	-
GM88	-	-	-	-	-	+	+	-
GM10	-	-	+	-	-	+	+	-
GM22	-	-	+	-	-	+	+	-
GM91	-	-	+	-	+	+	+	-
GM15	+	+	+	-	-	+	+	-
GM23	+	-	+	+	+	+	+	-
GM28	-	-	+	+	+	+	+	-
GM40	-	-	+	+	+	+	+	-
GM80	+	+	+	+	+	+	+	-
GM77	-	-	+	-	+	+	+	-
GM60	-	-	-	-	+	+	+	-
GM45	+	+	+	+	+	+	+	-
GM31	+	+	+	-	+	+	+	-
GM11	-	-	+	-	+	+	+	-
GM02	-	-	+	+	+	+	+	-
GM55	-	-	-	+	+	+	+	-
GM95	-	+	+	+	+	+	+	-
GM33	-	-	+	+	+	+	+	-
GM19	+	-	+	+	+	+	+	-
GM20	-	-	+	+	+	+	+	-
GM52	-	-	-		+	+	+	-
GM12	+	-	+	+	+	+	+	-
GM30	-	+	+	+	+	+	+	-
GM71	+	-	+	-	+	+	+	-
GM13	-	-	+	+	+	+	+	-
GM93	-	-	-	+	+	+	+	-
GM66	-	+	+	+	+	+	+	-

Group 1: GM5, GM11 and GM88. Group 2: GM10 and GM22. Group 3: GM91 and GM15. Group 4: GM23, GM28, GM40 and GM80. Group 5: GM77, GM60 and GM45. Group 6: GM31, GM07 and GM2. Group 7: GM55, GM95, GM33 and GM19. Group 8: GM20, GM52 and GM12. Group 9: GM30 and GM71. Group 10: GM13, GM93 and GM66

substances can have inhibitory lipid moiety in their chemical composition. These results suggest that the biochemical nature of the molecule produced is peptidic. The inhibitory compounds produced by the isolates showed great resilience to thermal treatments. In another way, bacteriocin has proved stable over a wide pH range with all peptides, now some antimicrobial activity in the pH range from pH 4-7. According to Allouche *et al*. [53]., recent bacteriocin is very sensitive to pH, its stability was detected at a pH range of 3.5 to 6.5. In this study, bacteriocin produced by isolates had the same profile and were active at pH values 4-6 (Table 6). In a similar study, the work of Zamfir *et al*. [44]. Reported that the bacteriocin produced by *L. acidophilus* develop a positive activity against *Staphylococcus aureus*.

CONCLUSIONS

Milk for human consumption must be properly collected from a healthy well fed female. The main causes of variations are related to race and species, but also depends on individual factors related to the health, nutrition and age of the animal. Quantitatively, cow milk is the raw material most widely produced and processed worldwide. However, the milk of other mammals - goats, sheep, buffalo and camel - is of significant importance in the economy of semi - arid regions and especially those of the Mediterranean basin. In Algeria, many traditional dairy products are not identified and studied; several types of cheeses are classified and identified in different parts of our country. Among these different types, we

Mentioned the following names Mechouna cheeses, Bouhezza, Madeghissa, Klila, Djben Takammerite, Aoules and Igounanes. In a variety of ecological niches, micro- organisms compete with each other for-survival and through evolution from unique flora. In some food ecosystems, lactic acid bacteria constitute the dominant microbiota of these bacteria widely distributed in the nature and occurring naturally as indigenous micro-biota in raw milk as Gram positive bacteria that play an important role in many foods and feed fermentations. These organisms are able to produce antimicrobial com-pounds against competing microbiota, including food-borne spoilage and pathogenic bacteria. They were considered as potential candidate lactic acid bacteria for use as starter culture in dairy milk fermented production.

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REFERENCES

1. Sfakianakis, P. and C. Tzia, 2014. Conventional and Innovative Processing of Milk for Yogurt Manufacture; Development of Texture and Flavor: Review, Foods, 3: 176-193.
2. Mechai, A. and D.D. Kirane, 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk (Raib). Afric. J. Biotech., 16: 2908-2914.
3. Ouadghiri, M., M. Amar, M. Vancanneyt and J. Swings, 2005. Biodiversity of lactic acid bacteria in Moroccan soft white cheese (Jben). FEMS Microbiology Letters, 251: 267-271.
4. Saranraj, P., M.A. Naidu and P. Sivasakthivelan, 2013. Lactic Acid Bacteria and its Antimicrobial Properties: A Review. International Journal of Pharmaceutical & Biological Archives, 4(6): 1124-1133.
5. Hendronoto Arnoldus, W.L., A. Lovita, S. Nooremma and L.B. Roostita, 2011. Isolation and identification lactic acid bacteria from raw beef meat. 3rd International Conference of Indonesian Societies for Lactic Acid Bacteria (3rd IC-ISLAB) the Faculty of Agricultural Technology, Gadjah Mada University, Indonesia January 21-22, 2011.
6. Lee, B., 1996. Bacteria-based processes and products. In: *Fundamentals of Food Biotechnology* VEH, B. Lee (Ed), New York, pp: 219-290.
7. Savijoki, K., H. Ingmer and P. Varmanen, 2006. Proteolytic systems of lactic acid bacteria. Appl. Microbiol. Biotechnol., 71: 394-406.
8. Zacharof, M.P. and R.W. Lovitt, 2012. Bacteriocins Produced by Lactic Acid Bacteria. Review Article. Biological & Environmental Engineering Society, 7(8): 2212-6708.
9. NAID, 2014. National Agency of Investment Development: Interview with DJELLAOUI Abd-el-Kader, Wali of Djelfa, Interview realized by ANDI with the Wali of Djelfa March 2014. 1-7.
10. BS 770-5., 1976. Methods for chemical analysis of cheese, Determination of pH value Cheese, Dairy products, Processed Foods, Food products, Food testing, chemical analysis and testing, pH measurement, Calibration. ISBN: 0 580 08747 6.
11. Burdikova, Z., Z. Svindrych, J. Pala, C.D. Hickey, M.G. Wilkinson, J. Panek, M.A.E. Auty, A. Periasamy and J.J. Sheehan, 2015. Measurement of pH micro-heterogeneity in natural cheese matrices by fluorescence lifetime imaging. Front Microbiol., 6: 183.
12. Guetouache, M., B. Guessas, S. Medjekal and O. Toumatia, 2015. Technological and Biochemical characterization of Lactic Acid Bacteria isolated from Algerian Traditional Dairy Products. World Applied Sciences Journal, 33(2): 234-241.
13. Tudor, L., L.I. Ilie, E. Mitrănescu, N. Ciocârlie and I. łogoe, 2009. The assessment of microbiological quality of some traditional Romanian cheeses. lucrări ştiinłifice medicină veterinară, XLII(2): 315-320.
14. Tasci, F., 2011. Microbiological and chemical properties of raw milk consumed in Burdur. Journal of Animal and Veterinary Advances, 10.5: 635-641.
15. Mata, G.M.S.C. and M.C.D. Vanetti, 2012. Comparison of conventional and rapid methods for Salmonella detection in artisanal minas cheese. Journal of Food Research, 1(3): 178-183.
16. Nabaraj, B., J.S. Mallory and E.H.A. Heather, 2015. Diversity of Yeast and Mold Species from a Variety of Cheese Types. Faculty Publications in Food Science and Technology. Paper 140. Current Microbiology, pp: 1-9.
17. GB 4789.15., 2010. National Food Safety Standard of P. R. China. Food Microbiological Examination: Enumeration of Yeasts and Molds. Issued Ministry of Health of P. R. China. ICS07.100.30. C53.
18. De Man, J., M. Rogosa and M.E. Sharpe, 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-135.

19. Terzaghi, B.E. and W.E. Sandine, 1975. Improved medium for Lactic Streptococci and their bacteriophages. Appl. Environ. Microbiol., 29: 807-813.
20. Mayeux, J.V., W.E. Andine and P.R. Elliker, 1962. A selective medium for detecting *leuconostoc* organisms in mixed-strain starter cultures. J. Dairy Sci., 45: 655-656.
21. Khedid, K., M. Faid, A. Mokhtari, A. Soulaymani and A. Zinedine, A. 2009. Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. Microbiological Research, 164: 81-91.
22. Schillinger, U. and F.K. Lucke, 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Envi Micro., 55: 1901-1906.
23. Thomas, T.D., 1973. Agar medium for differentiation of *Streptococcus cremoris* from the other bacteria. NZ J. Dairy. Sci. Technol., 8: 70-71.
24. Kempler, G.M. and LL. McKay, 1980. Improved medium for detection of citrate fermenting *Streptococcus lactis subsp diacetylactis*. J. Appl. Environ. Microbiol., 39: 956-927.
25. 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.
26. Forouhandeh, H., V.S. Zununi, M.S. Hejazi, M.R. Nahaei and M.D. Akbari, 2010. Isolation and Phenotypic Characterization of*Lactobacillus* Species from Various Dairy Products. Current Research in Bacteriology, 3: 84-88.
27. Durlu, O., V. Xanthopoulos, N. Tunail and E.T. Litopoulou, 2001. Technologically important properties of lactic acid bacteria isolates from Beyaz cheese made from raw ewes' milk. J. Appl. Microbiol., 91: 861-870.
28. Accolas, J.P., R. Bloquel and J. Regnier, 1977. Acidifying properties of the thermopiles lactic bacteria in relation to the manufacture of yoghourt, Milk, 67: 1-23.
29. Jini, R., H.C. Swapna, K.R. Amit, R. Vrinda, P.M. Halami, N.M. Sachindra and N. Bhaskar, 2011. Isolation and characterization of potential lactic acid bacteria (LAB) from freshwater fish processing wastes for application in fermentative utilisation of fish processing waste. Braz J. Microbiol., 42: 1516-1525.
30. Leuschner, R.G., P.M. Kenneally and E.K. Arendt, 1997. Method for the rapid quantitative detection of lipolytic activity among food fermenting microorganisms. International Journal of Food Microbiology, 37: 237-240.
31. Akabanda, F., J.O. Kwarteng, K.T. Debrah, C. Parkouda and L. Jespersen. 2014. The Use of Lactic Acid Bacteria Starter Culture in the Production of Nunu, a Spontaneously Fermented Milk Product in Ghana. International Journal of Food Science. ID 721067 1-11.
32. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical. Biochemistry, 72: 248-254.
33. Barefoot, S.F. and T.R. Klaenhammer, 1983. Detection and activity of lacticin B, a bacteriocin produced by Lactobacillus acidophilus. Appl. Environ. Microbiol., 6: 1808-1815.
34. Mathur, S. and R. Singh, 2005. Antibiotic resistance in food lactic acid bacteria-a review. Int. J. Food. Microbiol., 105: 281-295.
35. Guetouache, M. and B. Guessas, 2015. Characterization and identification of lactic acid bacteria isolated from traditional cheese (*Klila*) prepared from cow's milk. African Journal of Microbiology Research, 9(2): 71-77.
36. Keyvani, M. and Bolandi, 2015. Physicochemical and Organoleptic Properties of Lighvan Cheese Fortified with Protulaca Oleracea Seed Oil. Journal of Chemical Health Risks, 5(1): 21-27.
37. Mennane, Z., M. Faid, M. Lagzouli, M. Ouhssine, M. Elyachioui, E. Berny, M. Ennouali and K. Khedid, 2007. Physico-Chemical, Microbial and Sensory Characterization of Moroccan *Klila*. Middle-East Journal of Scientific Research, 2(3-4): 93-97.
38. Rhiat, M.H. Labioui, A. Driouich, Z. Mennane and M. Ouhssine, 2013. Preparation of the starter trial production of chesses (Jben) and (Klila) at laboratory scale. Food Sci. Qua. Manage., 13: 2225-0557.
39. Pacheco, F.P. and A.B. Galindo. 2010. Microbial safety of raw milk cheeses traditionally made at a pH below 4.7 and with other hurdles limiting pathogens growth. Current Research, Technology and education Topics in Applied Microbiology and Microbial Biotechnology. A. Méndez-Vilas (Ed), pp: 1205-1216.

40. Terzic-Vidojevic, A., K. Tonkovic, A.L. Pavunc, J. Beganovic, I. Strahinic, M. Kojic, K. Veljovic, N. Golic, B. Kos, N. Cadez, L. Gregurek, J. Suskovic, J. Raspor and L.L. Topisirovic, 2015. Evaluation of autochthonous lactic acid bacteria as starter cultures for production of white pickled and fresh soft cheeses. LWT - Food Science and Technology, 63: 298-306.
41. EC (European Commission), 2001. Overview of Microbiological Criteria for Foodstuffs in Community Legislation in Force.
42. Kirdar, S.S. and O. Kursun. 2011. Microflora and pathogen bacteria in traditional Anatolian Canak (*Pan*) cheese. Journal of Animal and Veterinary Advances, 10(19): 2511-2515.
43. Senbetu, D.T., 2014. Hazard Analysis of Cheese Provided for Consumers in Hawassa/Ethiopia. J Food Process Technol., 5(2): 297.
44. Hassanzadazar, H. and A. Ehsani, 2013. Phenotypic Characterization of Lactic Acid Bacteria Isolated from Traditional *Koopeh* Cheese. Global Veterinaria, 10(2): 148-152.
45. Orla-jensen, S., 1919. The lactic acid bacteria. Copenhagen. Andr. Fred. Host-Sen.
46. Azadnia, P. and N.A.H. Khan, 2009. Identification of lactic acid bacteria isolated from traditional drinking yoghurt in tribes of Fars province. Iranian J. Vet. Res., 10: 28.
47. Axelsson, L., 2004. Lactic Acid Bacteria, Microbiological and Functional Aspects: Classification and Physiology. Edited by Salminen, S., von Wright A., Ouwehand, A.C. Third Edition. Marcel Dekker Inc, New York, pp: 634.
48. Reginensi, S.M., M.J. González and J. Bermúdez, 2013. Phenotypic and genotypic characterization of lactic acid bacteria isolated from cow, ewe and goat dairy artisanal farmhouses. Braz J. Microbiol., 44(2): 427-430.
49. Gomes, B.C., F.G.B.D. De Melo and E.C.P. De Martinis, 2010. Dualistic aspects of Enterococcus spp. in foods. Current Research, Technology and education Topics in Applied Microbiology and Microbial Biotechnology. A. Méndez-Vilas (Ed), pp: 1119-1125.
50. Thomson, J., C.H. Gentry and Weeks, 1994. Metabolism des sucres par bactéries lactiques. In Derroissart. H. Luquet. FM. Ed. bactéries lactiques aspect fondamentaux et technologique. Uriage. Lorica, pp: 239-290.
51. Juillard, V. and J. Richard, 1994. Mixed culture in milk of a proteinase-positive and a proteinase-negative variant of Lactococcus lactis subsp. Lactis: influence of initial percentage of proteinase-positive cells on the rate of acidification. Lait, 74: 3-12.
52. Atanasovaa, J., P. Monchevab and I. Ivanovab, 2014. Proteolytic and antimicrobial activity of lactic acid bacteria grown in goat milk. Biotechnology & Biotechnological Equipment, 28(6): 1073-1078.
53. Allouche, F., N.A. Hellal and A. Laraba, 2010. Etude de l'activité antimicrobienne des souches de Lactobacilles thermophiles utilisées dans l'industrie laitière. Rev. Nat. Technol., 3: 13-20.
54. Zamfir, M., R. Cailewaert, P.C. Cornea, L. Savu, L. Vatafu and L. Devuyst, 1999. Purification and characterisation of a bacteriocin produced by Lactobacillus acidophilus IBB 801. J. Appl. Microbiol., 87: 923-931.