Technological and Biochemical characterization of Lactic Acid Bacteria isolated from Algerian Traditional Dairy Products

Article *in* World Applied Sciences Journal · January 2015

CITATIONS
0

READS
262

4 authors:

Guetouache Mourad
Université de M'sila
24 PUBLICATIONS **54** CITATIONS
SEE PROFILE

Guessas Bettache
University of Oran
52 PUBLICATIONS **280** CITATIONS
SEE PROFILE

Medjekal Samir
Université Mohamed Boudiaf- M'sila
38 PUBLICATIONS **66** CITATIONS
SEE PROFILE

Omrane Toumatia
École Normale Supérieure de Kouba
30 PUBLICATIONS **201** CITATIONS
SEE PROFILE

World Applied Sciences Journal 33 (2): 234-241, 2015
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2015.33.02.1384

Technological and Biochemical characterization of Lactic Acid Bacteria isolated from Algerian Traditional Dairy Products

[1]Guetouache Mourad, [2]Guessas Bettache, [3]Medjekal Samir and [4]Toumatia Omrane

[1,3,4]University Mohamed Bouadiaf of M'sila, Faculty of Science,
Department of Microbiology and Biochemistry 28 000, Algeria
[1,2]Laboratory of Applied Microbiology, Department of Biology,
Faculty of Sciences, University 1 Ahmed Benbella of Oran, Algeria
[4]Laboratoire de Biologie des Systèmes Microbiens (LBSM),
École Normale Supérieure de Kouba, Alger, Algeria

Abstract: Morphological, biochemical and technological characteristics were employed to identify lactic acid bacteria (LAB), isolated from traditional dairy products (Butter, Klila and Jben) was collected from different rural areas of the province of Djelfa. 277 lactic acid bacteria strains were isolated, with 170 (61.37%) of them belonging to lactic acid cocci others (38.63%) to the lactic acid bacilli. Their proportion were *Lb. plantarium* (13.72%), *Lb. fermentum* (09.75%), *Lb. acidophilus* (11.91%), *Lb. helveticus* (07.94%), *Lb. paracasei subsp tolerans* (06.14), *Lb. casei subsp casei* (08.66), *Lc. lactis subsp cremoris* (07.22%), *Lc. lactis biovar* (11.19%), *Lc. dicetyllactis* (10.11%), *Lc. raffinolactis* (08.30%), *Leuconostoc lactis* (03.61%), *Leuconostoc mesenteroides subsp cremoris* (01.44%). But these proportions were depending on the product tested as follows: Butter (14.08%), Klila (07.58%) and Jben (78.34%). Presumptive lactobacilli counts ranged from $1.5x10^{+3}$ to $3.4x10^{+8}$ cfu/ml, presumptive lactococci levels varied from $2.2.10^{+2}$ to $4.2.10^{+6}$ cfu/ml, presumptive Leuconostoc levels ranged from $2.8x10^{+1}$ to $7.2.10^{+3}$ CFU/ml. According to the method of Bradford (1976). Proteolytic strains GM99, GM27, GM31, GM10 and GM14 isolated from these traditional products have an average consumption rate of the casein equal to (833 µg/h, 820 µg/h, 809 µg/h, 530 µg/h and 216 µg/h), respectively. The proteolytic system involved in casein utilization provides cells with essential amino acids during growth in milk and is also of industrial importance due to its contribution to the development of organoleptic properties of fermented milk.

Keys words: Lactic acid bacteria · Bacilli · Cocci · Proteolytic · Casein · Traditional dairy products · Bradford

INTRODUCTION

Fermented milk provides the human diet with nutritious compounds of varied flavors, aromas, and textures. These products are based on the metabolic activity of lactic acid bacteria to ferment sugars, especially glucose and galactose, so to produce lactic acid and aromatic substances that give typical flavors and tastes to fermented products. Several types of fermented milk products have been reported to exist throughout the world [1]. In Algeria, the most popular are *Jben, Lben, Klila* and *Raib*. In a variety of ecological niches, micro-organisms complete with each other for survival through evolution from unique flora. LAB have played along an important role in food technology and have a long history of use by man for food production and preservation. A typical lactic acid bacterium grown under standard conditions (nonlimiting glucose concentration, growth factors and oxygen limitation) is gram-positive, nonsporing, catalase negative in absence of porphorinoids, aerotolerant, acid tolerant, organotrophic, and a strictly fermentative rod or coccus,

Corresponding Author: Guetouache Mourad, Department of Microbiology and Biochemistry, Faculty of Science, University of Mohamed Bouadiaf M'sila, 28 000, Algeria.

producing lactic acid as a major final product. It lacks cytochromes and is unable to synthesize porphyrins. The cells are usually nonmotile. They have a requirement for complex growth factors such as vitamins and amino acids [2]. These organisms are able to produce antimicrobial compounds against competing flora, including food-borne spoilage and pathogenic bacteria. In this group are included representatives of the genus *Lactobacillus*, *Enterococcus, Bifidobacterium, Aerococcus, Leuconostoc, Pediococcus, Streptococcus, Carnobacterium, Oenococcus, Weissella, Tetragenococcus, Vagococcus, Lactococcus* [3]. The lactic acid fermentation, which these bacteria carry out, has long been known and applied by humans for making different food stuff. In addition, they strongly determine the flavor, texture and, frequently, the nutritional value of food. They should possess stable fermentation characteristics and be resistant to bacteriophages [4]. The selected strains must have good biotechnological abilities, such as acidification, proteolytic and lipolytic activity. The proteolytic system involved casein utilization provides cells with essential amino acids during growth in milk and is also of industrial importance due to its contribution in development of fermented milk organoleptic properties . In milk fermentation processes, the proteolytic system of LAB plays a key role because for growth in milk. LAB are fastidious microorganisms that require an exogenous source of amino acids or peptides, which are provided by the proteolysis of casein, the most abundant protein in milk and the main source of amino acids. In general, the exploitation of casein by LAB is initiated by a cell-envelope proteinase (CEP) that degrades the protein into oligopeptides that are subsequently taken up by the cells via specific peptide transport systems for further degradation into shorter peptides and amino acids by a concerted action of various intracellular peptidases [5-6]. Proteolytic system is one of the most important biochemical processes involved in manufacturing of many fermented dairy products, for example in cheese production, 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]. The goal of this study was firstly, the isolation and taxonomic determination of large number of lactic acid bacteria from traditional fermented milk and secondly, the characterization of different microflora groups with good acidifying and proteolytic abilities.

MATERIAL AND METHODS

Samples Collection: The different samples studied traditional dairy products was collected from the rural area of the province of Djelfa. They were transported to the laboratory under refrigeration condition (4°C) and analyzed immediately; the pH measurement of the samples (sample preparation is carried out by dissolving 5 g solid dairy products in 25 ml of distilled water with a neutral pH, but for liquid dairy products, in aseptic conditions, one gram of traditional butter decomposed into 9 ml of 2% solution of potassium di-phosphate, pH 7.5 ± 0.1 sterile. So this batch is melted in a water bath not exceeding 45°C and agitating [8] is performed by a pH meter with an Orion Research type combination electrode previously calibrated with buffer solutions at pH 4 and pH 7. We transfer 10 ml of sample to a small beaker then we 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 [9].

Identification of Lactic Acid Bacteria Isolates: MRS agar and Broth were used for enumeration and culture of LAB. The MRS plates overlaid with MRS agar and incubated at 37°C for 48–72 h. Well - isolated colonies with typical characteristics namely pure white, small with entire margins were picked from each plate and transferred to MRS. 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 temperatures 15, 30, 37 and 45 °C; growth at different pH values; growth at different NaCl concentrations [10], Hydrolysis of arginine was tested in M16BPC [11]. 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 Mc Kay (1980) medium. Production of acetone from glucose was determined using Voges-Proskauer test [12]. For performing the biochemical tests, an MRS-BCP broth medium (BCP 0.17 g/l) was used. The carbon source was added to the sterile basal medium as filter sterilized solution to a final concentration of 1%. Carbohydrates utilization was assessed at the 24 and 48th h. All strains were tested for fermentation of the following 18 sugars: L-arabinose, D-xylose, galactose, D-fructose, mannitol,

sorbitol, cellobiose, maltose, lactose, mélibiose, tréhalose, mannose, rhamnose, esculine, sucrose, D-ribose, salicine and D-raffinose. To ensure anaerobic conditions, two drops of sterile liquid paraffine were placed in each tube after inoculation. The selected strains were stored at-80°C in 10% skim milk broth [13].

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 a 18h preculture [14]. 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 plates were incubated at 30°C for 48 h. After incubation, colonies were enumerated, recorded as colony forming units (cfu/ml). Only plates containing between 30 and 300 colonies were retained [15]. 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*. [16]. Using NaOH (N/9) in the presence of phenolphthalein indicator (1% in alcohol). As for the proteolytic activity residual proteins concentrations in the culture were estimated by a Coomassie G-250 binding procedure [17].

Assay of Residual Protein: Proteolytic activity was tested using Plat Count Agar PCA with 1% and 2 % (w/v) skim milk. The presence of clear zones around the colonies was recorder as positive activity. The strains with revealed a positive reaction in MRS with 1% skimed milk were considered as strains with a proteolytic activity [18]. Only strains which have a strong high proteolytic are activity used for determination of residual proteins i.e., the study of the digestion profile of the casein was in a carried out using a preculture of the strains tested. The method consisting by to inoculating a colony with 10 ml of MRS medium casein, after incubation at 30° C for 24 hours culture is centrifuge at 4000 rd/ min for 10 min at 4° C. The cell pellet is washed with 5 ml of the solution of phosphate buffer, after agitation and centrifugation the pellet is suspended in 3ml of phosphate butter; then inoculated in 100 ml of buffered MRS medium (1g/l) casein. After homogenization by agitation, culture is distributed at a rate of 10 ml per tube and incubated at suitable temperatures (Mesophilic at 30°C and thermophilic at 45 °C). The assay is performed according to the method of Bradford [17]. This method is based on the absorption of colorant Coomassie brilliant blue G-250 (CBBG) solution was prepared by placing 3.5mg of (CBBG) in a small amount of water and adding 3.5ml of 95% (v/v) ethanol and 7.9 ml of 85% (v/v) phosphoric acid. The volume was made up to 100ml with water, producing a solution with the following final concentrations of reagents: 0.0035% (w/v) CBBG, 3.5% (v/v) ethanol and 7.5% (v/v) phosphoric acid. The solution was shaken for 15min to improve CBBG solubilization. Then it was filtered twice and stored in an amber flask. A 100 mM phosphate buffer solution at pH 7.2 was prepared by dissolving 1.3 g of potassium dihydrogen phosphate in 50ml of water. The pH was adjusted with a 100 mM NaOH solution and the volume was made up to 100ml with water stock solution which prepared by dissolving 10.0mg of casein in 50ml of the previously prepared phosphate buffer solution. protein reference solutions (25, 50, 75, 100 and 125 µg/ml) were prepared by diluting 1.25, 2.50, 3.75, 5.00 and 6.25ml of the casein stock solution with phosphate buffer solution, adjusting each volume appropriately according to [19]. The values obtained from the tubes of the standard range possible to plot a standard right absorbance = f (quantity).With most protein assays, sample protein concentrations are determined by comparing their assay responses to that of a dilution-series of standards whose concentrations are known [20]. When protein in the sample binds to the dye the absorption maximum of the dye shifts from 465 nm to 595 nm, so by measuring the absorption of light at 595 nm using a spectrophotometer, one can assess the protein concentration. The responses of the standards are used to plot or calculate a standard curve. Absorbance values of unknown samples are then interpolated onto the plot or formula for the standard curve to determine their concentrations [21].

RESULTS AND DISCUSSION

Isolation and Identification of Lactic Acid Bacteria: The greater part of the total number of isolates was gram positive and catalase-negative. A total of 277 isolates from four samples could be identified and were divided into three genera: *Lactobacillus*, *Lactococcus* and

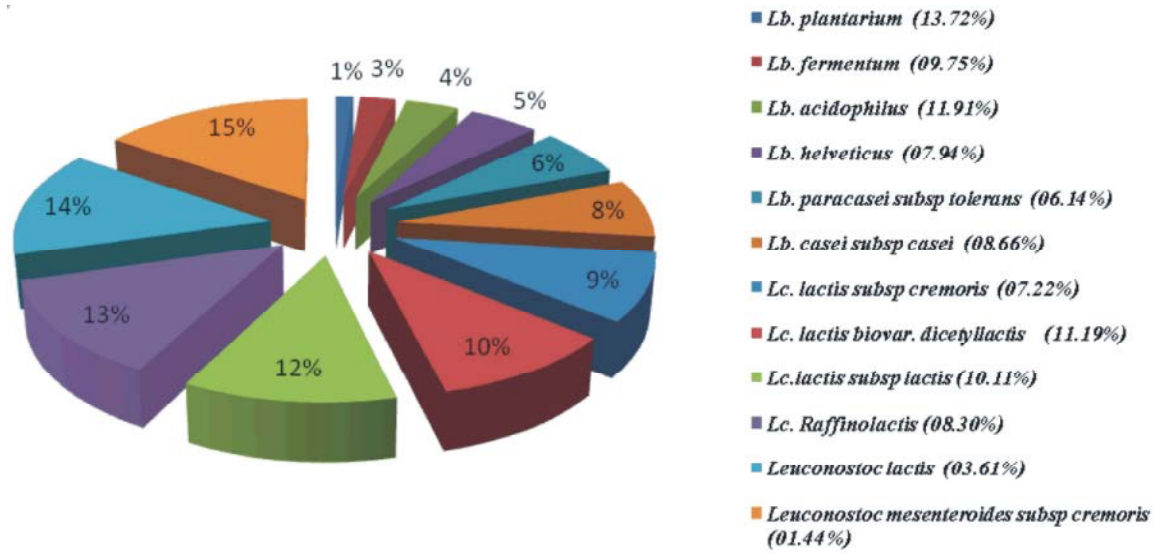

Fig. 1: Identity of 277 bacterial strains isolated from traditional dairy products.

Table 1: Morphological, cultural, physiological characteristics of Lactobacilli strains isolated from traditional dairy products

Species	GM99	GM27	GM17	GM38	GM24	GM22
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	+	+	-	+	+	+
- Melebiose	+	+	-	+	+	-
- Raffinose	+	+	-	+	+	-
- Ribose	-	+	-	+	+	-
- Lactose	+	+	+	+	-	+
- Rhamnose	-	-	-	+	+	-
- Sorbitol	-	+	-	+	+	+
- Xylose	-	-	-	-	+	-
- Tehalose	-	-	-	+	+	+
- Maltose	+	+	-	+	+	-
- Esculine	-	-	-	+	+	+
- Sucrose	+	+	-	+	+	-

GM38 Lactobacillus plantarium, GM22 Lactobacillus helveticus, GM99 Lactobacillus acidophilus, GM27 Lactobacillus fermentum and *GM17 Lactobacillus paracasei subsp. tolerans, GM24 Lactobacillus casei subsp. rhamnosus*

Table 2: Morphological, cultural, physiological characteristics of Cocci strains isolated from traditional dairy

Species	GM20	GM31	GM28	GM23	GM10	GM04
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	+	-	+	-	-	+
- Melebiose	-	-	-	+	-	-
- Raffinose	+	-	-	+	+	-
- Ribose	+	+	+	-	-	-
- Lactose	+	+	+	+	+	+
- Rhamnose	-	-	-	-	-	-
- Sorbitol	-	-	-	-	-	-
- Xylose	-	+	+	+	-	-
- Tehalose	+	-	-	+	-	-
- Maltose	+	+	+	+	+	+
- Esculine	-	-	-	-	-	-
- Sucrose	-	-	-	+	-	-
- Galactose	+	+	+	+	+	+
- *Fructose*	+	+	+	+	+	-

GM20 Lc. lactis subsp cremoris, *GM31 Lc. lactis biovar. dicetyllactis GM28 Lc. Lactis subsp. lactis*, *GM23 Lc. raffinolactis*, *GM10 Leuconostoc lactis*, *GM04 Leuconostoc mesenteroides subsp cremoris*.

Leuconostoc. Their proportion were *Lb. plantarium* (13.72%), *Lb. fermentum* (09.75%), *Lb. acidophilus* (11.91%), *Lb. helveticus* (07.94%), *Lb. paracasei subsp tolerans* (06.14%), *Lb. casei subsp casei* (08.66%), *Lc. lactis subsp cremoris* (07.22%), *Lc. lactis biovar. dicetyllactis* (11.19%), *Lc.lactis subsp lactis* (10.11%), *Lc. raffinolactis* (08.30%), *Leuconostoc lactis* (03.61%), *Leuconostoc mesenteroides subsp cremoris* (01.44%) (Figure 1).

The Lactobacilli group into three subgroups according to Orla-jensen [22-23] as follows: in our case into two types, *Lactobacillus plantarium are* mesophilic facultative hetero-fermentative, *Lactobacillus helveticus, Lactobacillus acidophilus, Lb. casei subsp rhamnosus, Lb. paracasei subsp tolerans* and *Lactobacillus fermentum* are thermophilic obligate homo-fermentative. The morphological, biochemical and physiological characterization of the isolates revealed that all the isolates that produced highest lactic acid among each group are *L acidophilus, L fermentum* and *L plantarium*. All isolates fermented the same carbohydrates; Olarte [24] noted that the presence of *L plantarium* in the cheese (Cameros) from goat's milk decreased the number of the enterbacteria and fecal Coliforms in the final product (Table 1). The rest of selected isolates were cocci with spherical or ovoid morphology and appeared mostly as pairs or forming chains therefore they tentatively referred to *Lactococcus* and *Leuconostoc*. The Leuconostoc are heterofermentative, but, unlike the similar Betabacteria, produce D (-) lactate and are unable to hydrolyze arginine to from ammonia. They have complex nutritional requirements and considered Vancomycine resistant according [25] (Table 2).

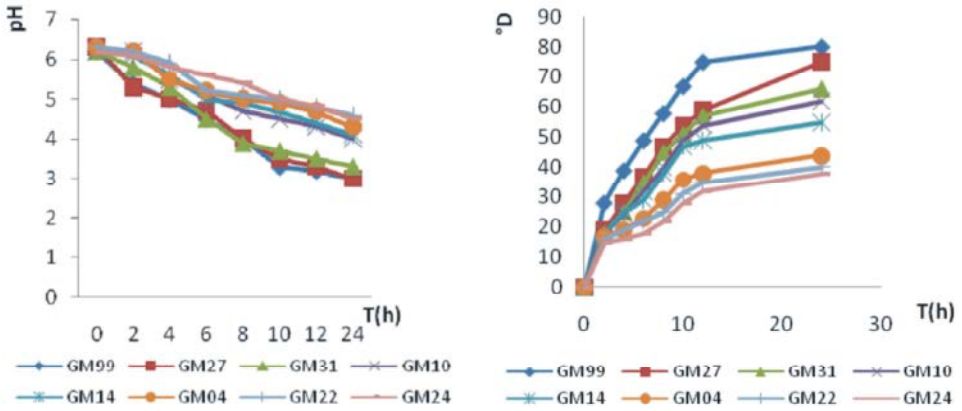

Fig. 2: Kinetics of degree Dornic acidity and pH of the different isolates in milk medium.

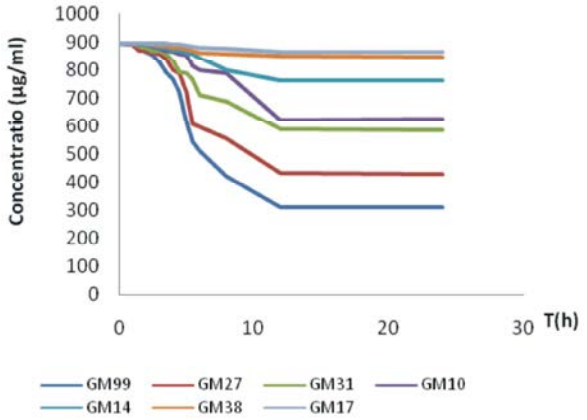

Fig. 3: Kinetics of use of caseins by the strains isolated in medium MRS casein with 30°C for the mesophilic and 45°C for the thermophilic.

Technological Study: The variation of acidification was monitored for all strains, as shown in Figure 2. The diminution of pH of the milk is due to the production of lactic acids from lactose fermentation [26]. 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 GM99, GM27, GM31, GM10 and GM14) that coagulate milk before 18 hours of incubation, low acidifying strains (strains GM04, GM22 and GM24) that coagulate milk after 18 hours of incubation and the remaining strains coagulate milk after 18 to 24 hours of incubation. The initial pH of skim milk was 6.2 to 6.4 for all the tested strains. Then, the pH decreases with time to reach 3.2-3.5 in highly proteolyses 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 81°D after 24 hours with strain GM33 and up of 38°D with the strain GM24.

Assay of Residual Protein: 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 [27]. The appearance of the zones of the proteolytic activity in the concentrations 1% and 1.5% is very easily detected. While in concentrations 3% detection is very low. In this case all the strains selected gave a zone of lyses on milieu PCA skim milk 1% with a variation of 01.8 mm to 07.9 mm. Strains GM99, GM27, GM31, GM10 and GM14 give a diameter of lyses of 07.9mm, 06.7 mm, 06.6 mm, 05.5 mm and 05.3 mm respectively. Thus they have a strong proteolytic activity (Figure 3). Preferably, one chooses the adequate concentration (lower than 2) to obtain strains with a great proteolytic power. The proportioning of casein obtained is illustrated in the graph of the figure. 4; from where one can say that the quantity of casein decreases quickly at the strains strongly proteolytic (GM99, GM27, GM31, GM10 and GM14) with a mean velocity of consumption equalizes with (833µg/h). And the reverse at the stocks slightly proteolytic (GM24, GM22, GM38 and GM17) with a mean velocity of consumption equalizes with (188 µg/h).

CONCLUSIONS

The lactic bacteria intervene in dairy industry and the fermentation of many other foods. They contribute in food texture and savor. It ferments glucids to lactic acid, which causes diminution of the pH used in food preservation. Their proteolytic power is necessary for a good growth in milk. The detection of the proteolytic activity was carried out on PCA medium added of 1% and 2% of skim milk, used to make easy the detection of proteolysis zones. The results of the residual casein evaluation provided us to follow the proteolysis kinetics of the following strains: *Lc. lactis biovar.dicetyllactis, Leuconostoc lactis, Leuconostoc mesenteroides subsp cremoris, Lactobacillus plantarium, Lactobacillus acidophilus, Lactobacillus fermentum and Lactobacillus paracasei subsp. tolerans*; in an optimal temperature (30 and 45°C). The *Lactobacillus acidophilus, Lactobacillus fermentum* and *Lc. lactis biovar. diacetylactis* strains are characterized by a quick consumption average of casein with 833mg/h. The proteolytic strains isolated from the traditional dairy products, can be exploited in Algerian dairy industry.

REFERENCES

1. Mechai, A. and D. Kirane, 2008. Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk (Raib). Afric. J. Biotech., 16: 2908-2914.
2. Axelsson, L., 2004. Lactic Acid Bacteria: Classification and physiology. In: Lactic Acid Bacteria, Microbiological and Functional Aspects. New. York, pp: 1-66.
3. Dortu, C. and P.H. Thonart, 2009. les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la bi-conversation des produits alimentaires. Biotech. Agron. Soc. Environ., 13: 143-154.
4. Lee, B., 1996. Bacteria-based processes and products. In: Fundamentals of Food Biotechnology VEH, B. Lee (Ed), New York, pp: 219- 290.
5. Kunji, E.R.S., I. Mierau, A. Hagting, B. Poolman and W.K. onings, 1996. The proteolytic systems of lactic acid bacteria. Antoine Van Leeuwenhoek, 70: 187-221.
6. Christensen, J.E., E.G., Dudley, J.A. Pederson and J.L. Steele, 1999. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie Van Leeuwenhoek, 76: 217-246.
7. Savijoki, K., H. Ingmer and P. Varmanen, 2006. Proteolytic systems of lactic acid bacteria. Appl. Microbiol. Biotechnol., 71: 394-406.
8. Boukrouh, N., 2005. Méthode d'analyse microbiologique du beurre, Journal Official de la république Algérienne N° 42, pp: 18.
9. 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 and Qua. Manag., 13: 2225-0557.
10. Schillinger, U. and F.K. Lucke, 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Envi Micro., 55: 1901-1906.
11. Thomas, T.D., 1973. Agar medium for differentiation of *Streptococcus cremoris* from the other bacteria. NZ J. Dairy. Sci. Technol., 8: 70-71.
12. 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.
13. Chen, Y.S., H.C. Wue and F. Yanagida, 2010. Isolation and characteristics of lactic acid bacteria isolated from Ripe Mulberries in Taiwan. Brazil. Jour. Microbi., 41: 1517-8382.
14. 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. Appli Microbiol., 91: 861-870.
15. 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.
16. 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.
17. 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.
18. 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.
19. Oseas da Silva, M.A. and M.A. Zezzi Arruda, 2006. Mechanization of Bradford reaction for the spectrophotometric determination of total proteins. Anatica. Bioch., 351: 155-157.

20. Sapan, C.V., R.L. Lundblad and N.C. Price, 1999. Colorimetric protein assay techniques. Biotechnol. Appl. Biochem., 29: 99-108.
21. Preejith1, P.V., C.S. Lim., A. Kishen., M.S. John and A. Asundi1, 2003. Total protein measurement using a fiber-optic evanescent wave-based biosensor. Biotech. Lett., 25: 105-110.
22. Orla-jensen, 1919. The lactic acid bacteria. Copenhagen. Andr. Fred. Host-Sen.
23. Moreik, K., 2011. Isolation of lactic acid-trlated bacteria from the pig mucosal proximal gastrointestinal trac, including Oisenella umbonata sp. Nov. and Veillonella Magana. Sp. Nov., 47: 8325-2789.
24. Olarte, C.S., E.G. Sanz and T.P. Fandas, 2000. The effect of a commercial starter culture addition on the ripening of an artisanal Goat's cheese (Cameros Cheese). J. Appl Microbiol., 3: 421-429.
25. Frank, C.J., D. Chill and N. Maida, 2002. The lactic acid bacteria: a literature survey. Crit. Rev. Microbiol., 4: 281-370.
26. Thomson, J., Gentry and C.H. 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.
27. 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.