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SANITARY QUALITY AND ECOLOGY OF THE LACTIC BACTERIA ISOLATED FROM TRADITIONAL BUTTER

GUETOUACHE MOURAD[1], GUESSAS BETTACHE[2] & TOUMATIA OMRANE[3]

[1,2]Laboratory of Applied Microbiology, Department of Biology, Faculty of Science, University D'Es-Senia Oran, Oran, Algeria

[3]Laboratory of Biology of the Microbial Systems (LBMS), Department of Nature and Life Sciences, Ecole Normale Superieure, Kouba, Algeria

ABSTRACT

Three samples of traditional butter were collected from the rural area (Djelfa). Were analyzed for their physicochemical parameters (pH and acidity) and microbiological. Butter is an easy to spread produced exclusively from milk and cream consists essentially of fat, water and a small amount of solids. After collection, the sample A1 (year 2012) of butter has investigated a charge of *Clastridium* (2.5 x 10^5 ufc / g), with a total absence of total coliforms, fecal coliforms and total aerobic mesophilic flora. Counts and identification of isolated samples from two traditional butter belonging to the region (A1 and A2) lactic acid bacteria; were performed on 42 strains isolated 20 strains have been identified and purified to 75% *Lactobacillus* and *Lactococcus* 25%.

KEYWORDS: Lactic Acid Bacteria, Butter, Isolation, Identification, Health

INTRODUCTION

Various types of fermented dairy products exist worldwide. Their nature depends on the type of milk used, pretreatment, fermentation conditions and subsequent treatment. The fermentation of milk primarily involves the lactic acid bacteria, Micrococci, the Coryneform and molds (Zamfir *et al*, 2006). Traditionally, the production of curd is dominated by actions of nature and results from the production of butter with curd are a co-product (Konte, 1997). The farmer butter obtained by churning fermented milk is washed, salted, kneaded. This highly appreciated by consumers for its taste and nutritional qualities of the product, is used as an additive in food products to boost the flavor and aroma of some traditional recipes (couscous, tagine, chicken) (Sakilid and Issoual 2003). Lactic acid bacteria are ubiquitous microorganisms that may be found in all types of habitat. They support human activity in everyday life, time that bacteria of the commensal flora of the gut flora or food plants. However, a common feature allows to unify into one large group: their ability to ferment carbohydrates to lactic acid (Matamoros, 2008). Lactic acid bacteria are useful to man microorganisms allowing it to manufacture and maintain many of his food. These bacteria are helpful to humans and contribute to the production of many fermented food products like cheese, bread, butter, etc. Currently, lactic acid bacteria include thirteen different bacterial genera: *Lactobacillus*, *Enterococcus, Bifidobacterium, Aerococcus, Leuconostoc, Pediococcus, Streptococcus, Carnobacterium, Oenococcus, Weissella, Tetragenococcus, Vagococcus, Lactococcus* (Dortu and Thonart, 2009).

MATERIALS AND METHODS

Three samples of butter were taken in the town of Djelfa in Algeria (Table 1). The butter was taken in sterile glass bottles and transported at ambient temperature in the dark until use.

Table 1: Origin of the Samples Used

Samples	Origin	Date of Preparation
Butter A1	Zone Rasse El Aine Djelfa, Elmalha	April 3, 2012
Butter A2	Zone Rasse El Aine Djelfa, Elmalha	April 11, 2013

The measured pH with a pH meter after calibration with pH 7.02 and 4.00 by immersion in a small volume of collected in a beaker (Labioui *et al*, 2009) melted butter. 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 (Boukrouh, 2005).

MICROBIOLOGICAL ANALYSIS

Sanitary Quality

Detection and Enumeration

The analyzes performed on traditional butter are the following:

- The principle of Yeast Counts based on the use of a medium made selective by addition of antibiotics such as glucose agar medium to said oxytetracycline (OGA). From decimal dilutions, introduce 1 ml aseptically in Petri dishes, and then pour the OGA medium, incubated at 25°C for 03-05 days (Ouali *et al*, 2003).
- The total aerobic mesophilic microflora is a good indicator of contamination is enumerated on Plate Count Agar (PCA) medium, incubated 24 hours at 30°C.
- Coliforms are wanted on agar (deoxycholate-citrate-lactose) (DCL) incubated 24 hours at 37°C for total coliforms and 44°C for fecal coliforms (Aggad *et al*, 2009).
- For *Salmonella*, a pre-enrichment on selenite-cysteine medium 12 hours is carried out at 37°C, followed on enrichment broth tetra-thionate 24 hours at 37°C and counting.
- For use in *Staphylococcus* mid Baird Parker added after the egg yolk and tellurite and incubated 48 hours at 37°C (Labioui *et al*, 2009). Staphylococci have the particularity to grow on the hyper-saline environments and species of *staphylococcus aureus* is able to ferment mannitol. Thus, staphylococci were counted on Mannitol Salt Agar medium containing NaCl and mannitol as the sole carbon source. 10^{-1} to 10^{-6} dilutions were seeded towards 1 ml of each dilution per box. Incubation is at 37°C for 24 hours to 48 hours. The characteristic appearances of colonies, golden yellow or orange are counted (Bouzaid *et al*, 2012).

STUDY OF LACTIC MICROFLORA

Count

For the enumeration, prepared beforehand a series of decimal dilution to 10^{-7} in the realization from a saline solution or peptone water. One milliliter of the last three dilutions (10^{-4}, 10^{-5} and 10^{-6}) is seeded deeply on media used.

Isolation and Identification of Strains of Lactic Acid Bacteria

Isolation of lactic acid from the traditional butter bacteria was done on MRS (de Man *et al*, 1960) and solid solid M17 medium (Mathot *et al*, 1994), the cultures were incubated 24 hours at 30°C in Petri dishes in the dark. Purification of the strain on MRS agar is performed by the method of streaking until obtaining identical pure colonies. The purified isolates were differentiated by the Gram stain, catalase and oxidase:

- Gram staining was performed according to the protocol described by Prescott *et al*, 2003. It used to classify bacteria according to their Gram, their cell morphology and association mode.
- Catalase test was performed according to the experimental protocol described by Prescott *et al*, 2003 This test is to put a colony taken from hydrogen peroxide, bubbling gas means that there's production enzyme catalase and the test is positive.
- Test oxidase was determined according to the protocol described by Kovacs *et al*, 1995. A pure colony taken MRS agar is placed on Whatman paper soaked oxidase reagent, the development of a blue color means that the test is positive which means that the isolate has the oxidase enzyme.

Only isolates Gram-positive, catalase and oxidase negative were considered lactic acid bacteria. For good continuity of work, isolates must be well preserved. Two conservation techniques are used, the first is for a short duration, it is by growing strains on MRS or M17 solid bow in sterile test tubes. After incubating the strains stored at 4°C (Kihal 1996). But the second is carried out for a long term preservation is carried out in skim milk and 30% glycerol and stored at - 80°C, to be prepared beforehand using the pellet (after incubation in optimal conditions, the centrifugal strains seeded in liquid medium at 3000 rev / min for 3 min) (Mathot *et al*, 1994). Production of arginine dihydrolase (ADH) by using the isolates is arginine Research agar M16BCP Thomas (1973). This medium allows us to differentiate between subspecies of *Lactococcus lactis* and *Lactococcus lactis*. *Subsp. Lactic* (ADH +) and (ADH). This test also allows us to identify the lactobacilli (ADH +) and (ADH -). Use of arginine occurs by maintaining the original color of the medium. A negative result will cause a color change to yellow.

The production of acetoin is sought on Clark and Lubs medium. After seeding inbred strains in 10 ml of this medium and after incubation, the reaction is carried Proskaver Voges, adding 5gouttes reactive α-naphthol in 6% absolute alcohol (VPI) and 5gouttes soda (NaOH) 16% in distilled water (VPII). The tubes are then shaken and left 10 min at room temperature. The production of acetoin results in the appearance of a pink ring on the surface of the medium (Badis and Lebrese, 2003; Guessas, 2007). The metabolism of citrate has long been known among *Lactococcus Lactis. Subsp. Lactus biovare diacetylactis* and that is its ability to fermented citrate. It is found in other bacteria. This test is performed on the medium Kempler and McKay (1980) containing ferric citrate and potassium ferrocyanide. The strains capable of fermenting citrate in the medium which allows the reaction between ferric ion and potassium ferricyanide. This reaction results in the formation of blue colonies with a blue center (after 18 - 48 hours of incubation) the strains unable to ferment citrate remain white. This test has a particular interest in the differentiation of subspecies of *Lactococcus*. Growth in the presence of sodium chloride is produced by seeding strain (young culture) in MRS medium supplemented with lactose 4%, 10% and 12% sodium chloride, incubating at 30°C for 24 hours.

The purpose of this test is to differentiate the types of lactobacilli (Frank *et al*, 2002), but using 6.5% NaCl.

Instead enterococci, lactic acid bacteria are sensitive to salt hyper medium (Mathara *et al*, 2004). Therefore, the ability to grow on the medium used in the presence of NaCl at different concentrations and at different pH values was observed for 2 to 3 days of incubation (Bedi *et al*, 2005). The growth test at 30°C and 45°C is performed for the strains to be identified; they are grown on MRS broth. The growth is demonstrated by the presence of disturbances. According to the optimal growth temperature, bacteria are classified as mesophilic whose temperature is 30°C and thermophilic with optimal growth temperature of 40°C (Frank *et al*, 2002). Test fermentation type isolates isolated from lactic acid bacteria from traditional butter used to assess the type of fermentation by the carbon substrate which is converted.

It is also to highlight the formation of gas (CO2). It is carried out by incubating the strain in a MRS medium containing glucose Durham inverted bell, which allows the identification of the product gas, the development of hetero-fermentative bacteria is manifested by the occurrence of gas in the bell Durham and which is absent in homo-fermentative bacteria (Sembene, 2002). The study of the fermentation of the sugars is carried out on the medium to each specific kind of conventional tubes galleries on M17 and MRS liquid medium containing bromcresol purple (0.04 g / l) as a pH indicator and supplemented with 1% carbohydrate (Badis *et al*, 2005). The different types of sugars are used: rhamnose, glucose, xylose, mannitol, maltose, fructose, cellobiose, raffinose, galactose, mannose, and ribose.

RESULTS AND DISCUSSIONS

Physicochemical Analysis

The pH ranges from traditional butter 4.55 to 5.87 with an average of 5.08. Mean titratable acidity value of 16% (Table 2).

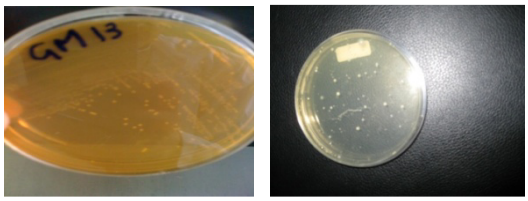
Table 2: Results of the Physicochemical Analysis of the Traditional Butter Sampling Different Regions

	Samples					
	A 1			A 1		
	E1	E2	E3	E4	E5	E6
pH	4.8	4.55	5.28	4.75	5.25	5.87
°D	23	28	11	15	12	07

D: The Dornic acidity, the acidity is expressed in degrees.
° Dornic or D 1 = 0.1 g / l of lactic acid.

Microbiological Analysis

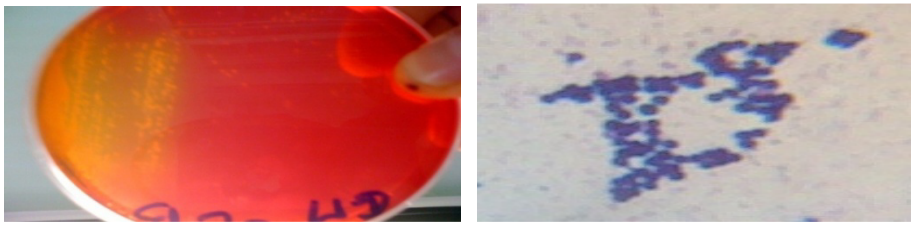
The results of the census conducted on samples of traditional butter of the region (A1 and A2) are listed in Table 3 These methods do not take into account that living organisms, they allow the use of selective media, and therefore specific counts (Figure 1).



A B

Figure 1: Appearance of Colonies of Lactic Acid Bacteria Obtained after Incubation of 24 to 30°C on MRS (A: After Isolation Streak Seeding, B: Seeding Depth) Medium

The analysis of these results shows the presence of notable variations between samples studied butter samples examined contain a variable load of total aerobic mesophilic flora (FMAT) between 1.3. 10^{+6} and 4.1.10^{+6} cfu / g of butter, the analysis said contamination of the samples A1 (E1, E2 and E3) in total coliforms with values of 0.8.10^{+5}, 2.2. 10^{+5} and 3. 10^{+5} cfu / gr respectively, with fecal coliform values 1.3. 10^{+4}.4.4. 10^{+4} 5.2. 10^{+4} and 4.4$10^{+4}$ cfu / g and contain respectively a variable charge of *Staphylococcus* (Figure 2), according to the following values: 2.1. 10^{+3}+ 3 1.2. 10^{+3} and 0.0 cfu / g respectively. However, all samples were free of *Salmonella* (Table 3). The study on indicators of microorganisms of fecal contamination can judge the hygienic condition of the product. Even at low levels, they would demonstrate hygienic conditions deteriorated during milking or transport yard (Labioui *et al*, 2009), the results obtained from the region on the sample A1 (E1, E2 and E3) indicates that butters studied are highly contaminated; they lose their capacity to become dangerous products to consumers, but the burden of yeasts 10.4. 10^{+2} cfu / g to 22.1. 10^{+2} cfu / g is normal to standard.



A B

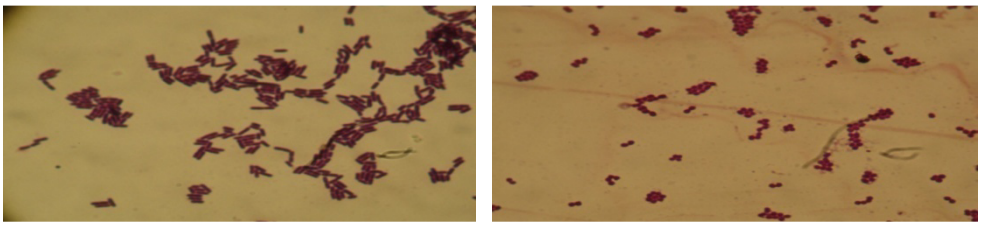
Figure 2: A: Appearance of Colonies of *Staphylococcus* Obtained after Incubation for 48 h at 37°C on Chapman Medium, B: Microscopic Observation of *Staphylococcus* (X1250)

Samples A2 Area (E4, E5 and E6) traditional butter tested exhibit relatively good microbiological quality and are acceptable from the hygienic point of view, lack of *Salmonella* and Staphylococcal indicates good health and animal a healthy trafficking and manufacturing. The average load of lactic bacteria (total microflora microflora acidifying, proteolytic microflora, *Lactobacillus* and *Leuconostoc*). By samples of 04, 3.10^{5} cfu / g, 03,6.10^{3} cfu / g, 02,6.10^{6} cfu / g, 02,7.10^{4} cfu / g and 01,3.10^{2} cfu / g, respectively.

Table 3: Results of Microbiological Analyses (Cfu / G) of Traditional Butter from Different Parts of the Levy

		Samples						M
		A1			A2			
		E1	E2	E3	E4	E5	E6	
Sanitary Quality	Yeast 10^{+2}	12	10.4	18.6	12.9	18.4	22.1	15.7
	Total aerobic mesophilic microflora. 10^{+6}	4.1	2.3	1.3	0.0	0.0	0.0	01.3
	Coliforms							
	Total coliforms. 10^{+5}	0.8	2.2	3.0	0.0	0.0	0.0	01.0
	Fecal coliform. 10^{+4}	1.3	4.4	5.2	0.0	0.0	0.0	01.8
	Staphylococcus. 10^{+3}	2.1	1.2	0.0	0.0	0.0	0.0	00.6
	Salmonelles.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lactic Microflora	Total microflora. 10^{+5}	4.2	3.6	2.8	4.8	5.2	5.4	04.3
	Microflora acidifying. 10^{+3}	2.1	1.8	3.5	4.5	4.1	5.8	03.6
	Microflora proteolytic. 10^{+6}	2.4	2.8	3.1	4.2	4.6	4.3	02.8
	Lactobacillus. 10^{+4}	3.5	3.9	4.2	1.3	1.4	2.2	02.7
	Lactococcus. 10^{+2}	0.0	0.0	1.6	2.7	3.4	0.0	01.3

Among the 40 strains isolated from traditional butter to two years (A1 and A2 2012 2013), on the media used at temperatures of 28°C and 45°C in aerobic conditions and 20 strains anaerobic Gram-positive and catalase were selected and studied. Microscopic observation (cells, Figure 3) and macroscopic (colonies, Figure 1) revealed two forms of well-isolated cells, circular and lenticular forms of creamy white color. The rods are 75% of the total are represented by the genus *Lactobacillus*. The hulls forms observed are represented by the *Lactococcus* with 25% of the strains.



A **B**

Figure 3: Represents the Microscopic Observation of Lactic Acid Bacteria (A: *Lactobacillus*, B: *Lactococcus*) (X1250)

20 strains of lactic acid bacteria are considered Gram positive, motionless, catalase negative. Biochemical identification of these strains of lactic acid bacteria 06 different bacterial groups of the genus *Lactobacillus* (*Lac. Plantarium casei. Lac. Rhamnosus lake. Fermentum lake. Acidophilus and Lake Lake. ssp. Casei. Helveticus*). And 03 different bacterial groups of *Lactococcus* (*L. lactis, L. and L. cremoris raffinolactis*). The distribution of different types are represented in the figure (4).

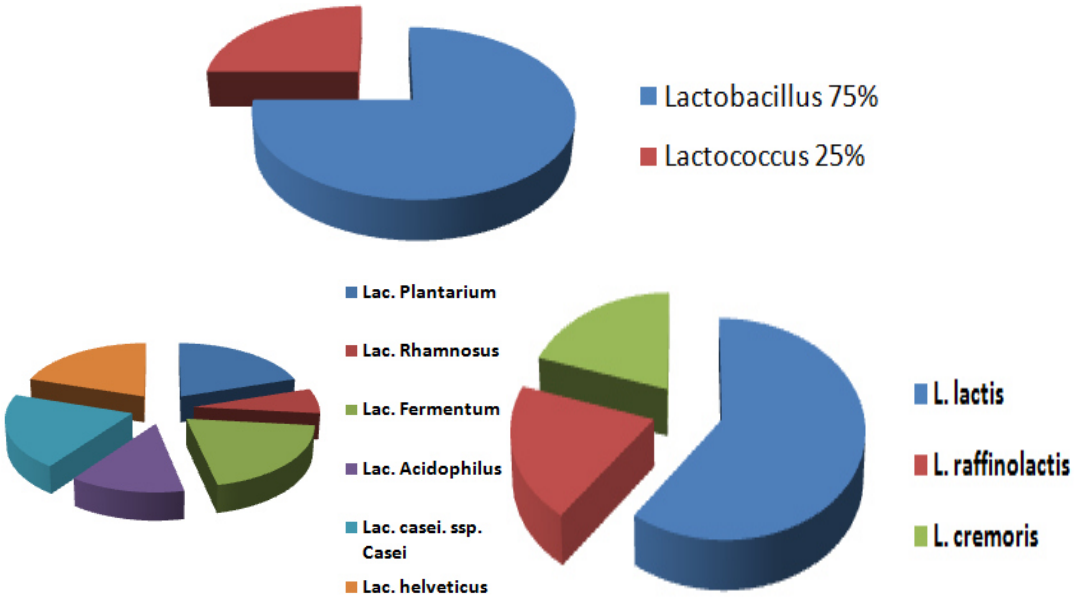


Figure 4: Distribution of the Different Groups of Lactic Acid Bacteria Isolated from Traditional Butter

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