



Characterization of Natural Lactic Flora in a Soft Cheese “Camembert of the Tessala” Made From Thermised Milk of Local Breed Cow “Brown of the Atlas”

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ABSTRACT

Microbial communities play an important role in the maturation of cheese and determine to a large degree its taste quality. The typicality and the sensory richness of Tessala camembert diversity is preserved by this microflora. In the present study, we tried after isolation and purification to characterize genotypically lactic microflora of this cheese at the end of maturation of the transformation from thermized milk of local breed cow. The bacterial DNA from twenty-two purified lactic culture was established by an amplification of ribosomal DNA 16S by specific universal primers of prokaryotes with strains reference of each isolated bacterial species used as positive controls. The results show the characteristic of the Tessala camembert by the diversity of its original lactic flora dominated by lactococci, enterococci, lactobacilli, pediococci and leuconostocs, preserved by the initial thermization of milk used to maintain the technologically-interested dairy microbial ecosystem.

Key words: Camembert of the Tessala, Characterization, Lactic microflora.

INTRODUCTION

The structure of a microbial community of cheese is appreciated by its species composition, intraspecific diversity and relative abundance of the various species. This microbial flora, called natural or indigenous, plays an important role in the quality of cheeses from raw or just moderately processed milk by a simple thermization, especially with regard to taste. It allows preserving the typical characteristics and sensory diversity of cheeses (Jadhav *et al.*, 2008, Monnet, 2015, Sakore *et al.*, 2007 and Shekhar *et al.*, 2010).

In Algeria, cow's milk is transformed into soft type camembert cheese either traditionally or industrially. The products of a traditional technology retain their desirable qualities even after long storage at room temperature because there are technological skills developed by natural microflora of milk (Aissaoui, 2006).

Traditional cheese hosts a diversified microbiota composed of endogenous microbial populations, which plays a major role in the development of the nutritional and organoleptic qualities (Leclercq, 2011).

This diversity of the lactic microflora will be specified, in this study, with the characterization of lactic isolated bacterial ecosystem of Tessala in ripening-camembert, produced by traditional technology; by way of culture on selective isolation and identification of environments and genotypic methods, by quantitative PCR and by chromatographic analyses of genomes fragments of the isolated lactic bacteria.

MATERIALS AND METHODS

Origin of cheeses

Studied cheeses were from 03 livestock farms in the west of Algeria, made in spring (from March 15 to June 15) and

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referenced from the same region of Sidi Lahcene municipality, wilaya of Sidi Belabbes, one month apart. Production and refining were carried out according to the usual procedure of the cheese factory “Tessala”, from thermized local breed cow milk “Brown of the Atlas” at 63°C, in 03 livestock farms and without sowing in lactic flora.

The samples of the camembert of the Tessala

Samples of cheese, in late maturation on the 10th day of refining, were collected aseptically in sterile containers of Tessala factory, kept at low temperatures and expedited to laboratory for experimental tests.

Microbiological tests

Ten grams of mass of camembert cheese were taken aseptically, placed in 90 ml of a sterile vial physiological water, mixed in a grinder and incubated at 37°C for 24 hours. The separation of the two phases were then carried out by

centrifugation for 10 minutes at 3500 rpm. The separate clear phase represents the 10^{-1} dilution parent solution. By using a sterile pipette, 1 ml of the aqueous phase 10^{-1} dilution is taken and introduced into a tube containing 9ml of sterile physiological water, thus 10^{-2} dilution is obtained. Dilution 10^{-3} to 10^{-6} are obtained in the same way (O'Sullivan *et al.*, 2015).

The lactic bacteria count

The media used for the culture of lactic bacteria were as follows:

–**MRS medium:** This medium Man, Rogosa and Sharpe “MRS” enriched culture of all species of *Lactobacillus* by lowering the pH to 5,7 and the addition of 0,14% sorbic acid (Dubernet *et al.*, 2008).

–**M17 medium:** This culture medium is recommended for the lactic flora in hull (Jany *et al.*, 2008).

The inoculation of plates is done in the mass of the media and are incubated at 30°C, 37°C and 45°C for 48 hours to 72 hours. Counts of bacterial groups search was done using a counter of microbial colonies (New Brunswick scientific CO Model C-1006327). The results are expressed in numbers of cells per ml (Fricker *et al.*, 2011).

Isolation and purification

After count, apparent characteristic colonies of bacterial groups were taken from plate for the study of their morphology.

The bacteria, in spherical and rod-shaped form, gram positive, catalase negative, not producing release of oxygen when they were separated on a drop of oxygenated water, are retained as lactic bacteria. A sample among the well isolated and purified colonies appearing on plate was taken and stored at 4°C with the MRS medium and 15% of glycerol in eppendorf tubes to identify the species of isolated lactic bacteria (Irlinger *et al.*, 2015).

Genotypic characterization of purified isolates

Identification of the isolates purified by phenotypic methods was not reliably identified (Reats *et al.*, 2011). There were developed methods at the level of the laboratory, a tool

based on molecular biology with an amplification of ribosomal DNA 16s, by universal primers specific to prokaryotes, a critical analysis of the resulting sequence, and a comparison with the positive control of species. *Enterococcus faecalis* ATCC 14506, *Enterococcus faecium* ATCC 27270, *Lactobacillus acidophilus* ATCC 4356 and DSM 20079, *Lactobacillus casei* ATCC 393, *Lactobacillus delbrueckii* ATCC 11842, *Lactobacillus fermentum* ATCC 9338, *Lactococcus lactis* ATCC 49032, *Leuconostoc mesenteroides subsp. cremoris* ATCC 19254 and *Pediococcus acidilactici* ATCC 8042.

RESULTS AND DISCUSSION

Enumeration of lactic bacteria

Lactic bacteria have been counted in all studied samples of cheese and in high phase of lactation of local cow milk. Their number varies from 1.9×10^2 cfu/g to 2.3×10^2 cfu/g, for one isolate in the MRS environment and 2.5×10^5 on 4.5×10^5 cfu/g on the selective medium M17. This difference in number of lactic bacteria in the samples is a result of the variability of the dairy microbial ecosystem within the same local cow breed “the Brown of the Atlas” with predominance to 38% lactococci, 29% lactic enterococci, 19% Lactobacilli, 10% pediococci and 4% leuconostocs. The number determines the quality of sampling in relation to the breed of the same farm and transformation as described by Delbes *et al.* (2015).

Isolation and purification

In this study, we are interested only in lactic bacteria isolated at the end of ripening of camembert to the 10th day of refining. 22 genera of lactic bacteria isolates were selected; 08 isolates of lactococci, 06 isolates of lactic enterococci, 05 isolates of Lactobacilli, 02 isolates of pediococci and 01 isolate of leuconostoc. Physiological and biochemical tests have shown that all isolates were gram positive and catalase negative which is a characteristic of lactic bacteria. Fig 1 shows the microscopic appearance of different isolated lactic strains.

Table 1: Impact of lactic bacteria in the traditional cheese of soft dough type “the Tessala camembert.

Microbiological analysis	1 st Farm Milk	2 nd Farm Milk	3 rd Farm Milk	1 st Farm Cheese	2 nd Farm Cheese	3 rd Farm Cheese
Total flora cfu/ml or g	2×10^8	1.5×10^8	1.7×10^8	2×10^6	1.5×10^6	1.8×10^6
Lactic flora on MRS cfu/ml or g	2×10^4	8×10^3	1.5×10^4	2.3×10^2	1.9×10^2	2.1×10^2
Lactic flora on M17 cfu/ml or g	8×10^5	4×10^5	6×10^5	4.5×10^5	2.5×10^5	3.5×10^5

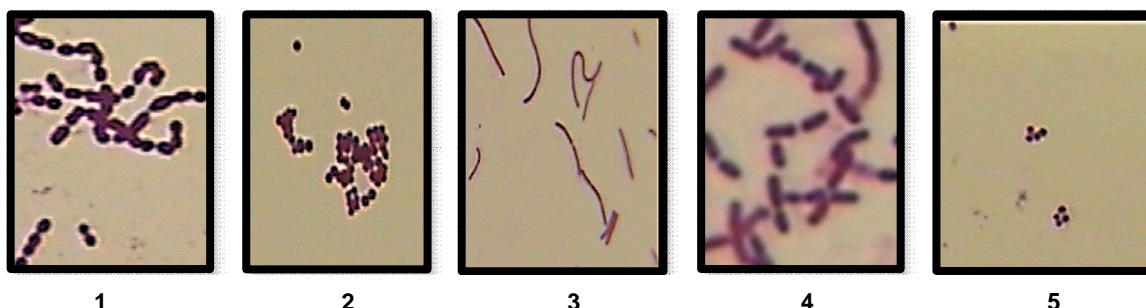


Fig 1: Microscopic aspect character of the isolated lactic strains (Immersion microscopic observation x1000).

1: Lactococci, 2: Lactic Enterococci, 3: Lactobacilli, 4: Leuconostoc, 5: Pediococci

Table 2: Morphological characters of genera of lactic strains.

Macro-morphology	Micro-morphology	Temperature°C	Groups
Round or lenticular white colonies	Cocci diplocoques and in chains	37 to 45°C	Lactic enterococci
Round or lenticular white colonies	Cocci diplocoques and in chains	25 to 37°C	Lactococci
Very small and round transparent colonies	Oval cocci in chains	15 to 37°C	Leuconostoc
Round or lenticular small white colonies or bulging Brown Center	Small sticks and in chains	37 to 45°C	Lactobacilli
Smooth rounded greyish or whitish colonies	Cocci in tetrads	25 to 37°C	Pediococci

Table 3: Physiological and biochemical of isolated lactic stem profile.

The strain characters	Coloring Gram	Form	Catalase	Fermentation type	Growth at 30°C	Growth at 37°C	Growth at 45°C
Enterococci	+	Hull	-	Hetero	-	+	+
Lactobacilli	+	Stick	-	Hetero	-	+	+
Lactococci	+	Hull	-	Homo	+	+	-
Leuconostoc	+	Hull	-	Hetero	+	+	-
Pediococci	+	Hull	-	Hetero	+	+	-

Table 4: Identification of the lactic bacteria isolated from cheeses in high lactation by the technique of the ARNr16s.

Strains of lactic bacteria	22 species identified	Molecular Identification	% I.D	marking band DNA
Lactococci	4	<i>Lactococcus lactis subsp cremoris</i>	100	320 BP
Lactococci	4	<i>Lactococcus lactis</i>	100	380 BP
Leuconostoc	1	<i>Leuconostoc mesenteroides subsp cremoris</i>	100	610 BP
Pediococci	1	<i>Pediococcus acidilactici</i>	100	810 BP
Pediococci	1	<i>Pediococcus pentosaceus</i>	100	720 BP
Lactobacilli	1	<i>Lactobacillus delbrueckii</i>	100	480 BP
Lactobacilli	2	<i>Lactobacillus acidophilus</i>	100	510 BP
Lactobacilli	1	<i>Lactobacillus fermentum</i>	100	540 BP
Lactobacilli	1	<i>Lactobacillus casei</i>	100	580 BP
Enterococci	4	<i>Enterococcus faecalis</i>	100	840 BP
Enterococci	2	<i>Enterococcus faecium</i>	100	890 BP

% I.D : % identification

BP : Base pairs

Characterized lactic strains provided a profile of different fermentation (Table 2):

-hetero-fermentative for lactic enterococci, lactobacilli, leuconostoc and pediococci

-homo-fermentative for lactococci

Bacterial counts from camembert cheese with local breed cow milk, which is produced in 03 farms in the same region, had an excellent repeatability for the studied lactic flora (Table 1). The standard deviation of experimental repeatability amounted to 0.5 cfu/g for the total flora, 0.2 cfu/g for the lactic flora isolated on MRS and one cfu/g for the lactic flora characterized on medium M17. Population levels are comparable to those that have been observed on similar cheeses made from raw milk (Manzo *et al.*, 2019 and Dubernet *et al.*, 2008), but comparably different due to heat treatment of thermization applied on our processed milks.

This bacterial count found a dominance of the lactic

flora on the M17 medium with respectively a number ranging from 4×10^5 to 6×10^5 then 8×10^5 cfu per ml on milks, and cheeses availability graded from 2.5×10^5 , to 3.5×10^5 and 4.5×10^5 cfu per gram.

The proportions rate of the different bacterial groups (on selective media MRS and M17) are eminently variable from milk to another, in relation to the variety of operating environments.

The counted proportion at the levels of lactic bacteria's on two selective media, used in cow milk of the same breed and a cheese pie and the same technology. The size of the herd, feeding the animals, housing (litter bedded or not) but above all trafficking practices (washing of teats before milking, post soaking, cleaning of the milking machine), are elements that, more or less combined determine the composition and structure of the bacterial communities in the milk and therefore manufactured cheeses. These results were consistent with those of Montel *et al.*, 2012.

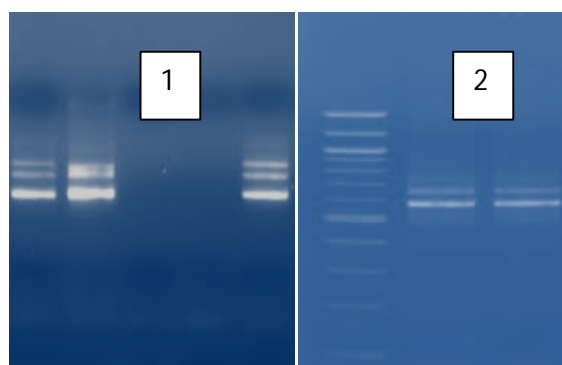


Fig 2: Electrophoresis gel of PCR products (Dominant lactic cheese isolate: *Lactococcus*).

1- At 320 base pairs *Lactococcus lactis* subsp *cremoris*.

2- At 380 base pairs *Lactococcus lactis*.

Knowledge of the diversity of the lactic flora of the Tessala camembert cheese was first acquired by methods of isolation, identification and purification of 22 isolates based on cultures with specific media. The lactic bacteria isolated form a heterogeneous group made up mostly of cocci and rod-shape, which main characteristic was the production of lactic acid, no pathogens, these bacteria to positive (Gram+) gram stain have an optional anaerobic metabolism and did not produce catalase (Table 3).

Taxonomic analysis, finer using molecular methods supported by DNA sequencing techniques PCR, confirmed the results obtained by Depouilly *et al.* (2004) and Quigley (2011). Indeed, the genotypic study carried out by PCR of profiles in different molecular weights (in BP) comparable to those of used strains of references (Table 4) is a characteristic of DNA bands for 22 species visualized positively after electrophoresis on 2% agarose gel. Fig 2 shows the electrophoretic profile of PCR products obtained from the DNA of dominant species on the Tessala camembert: *Lactococcus*.

By comparing these results to those of the bibliography (Casalta, 2003 and Monnet, 2015), in cheeses, soft type camembert made with local cow milk. acidophilic lactic bacteria remain best adapted to the conditions prevailing in cheese dough of experimental samples, homofermentaires, lactic enterococci, lactococci and heterofermentative lactobacilli dominate during traditional processing with proteolytic activity, promoting the production of growth factors amino acids necessary for the development of the lactic microflora, secondary dependent on including the leuconostocs and pediococci.

Various similar studies confirm the results obtained and highlight the role of the abundance and diversity of the microbiota of milks moderately covered in the preservation of the microbial ecosystem of cheeses and the formation of the very typical organoleptic aspects.

CONCLUSION

This established research has confirmed that the natural microflora plays an important role in the quality of traditionally

manufactured cheeses and manufactured thermized cow milk, especially on the organoleptic plan. Cheese ecosystem would not work without microflora.

This microflora represented mainly by the lactococci and Lactobacilli and lactic enterococci helps preserve the typicality by their technological skills confirmed either by their especially proteolytic enzyme activity which gives to the the Tessala Camembert significant nutritional value by the essential amino acids released by the acidophilic flora. Furthermore, in industrialized countries, the practice of cleaning and disinfection of the teats to the Treaty have improved the hygienic quality of raw milk and concomitantly reduced its microbial burden and thus influenced the microbial ecosystem natural dairy cheeses. Species of lactic bacteria, which have become rare in these countries, are frequent in artisan made cheeses of the region, which contribute to the enrichment of the niche and the knowledge of their ecology.

Moreover, thanks to the technological developments of the last decade, we have started to get a reliable picture of the microbial diversity of cheeses made from the milk of local livestock that we have to exploit to the standardization of the sensory characteristics of our cheeses.

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REFERENCES

- Aissaoui, Z.O. (2006). Making and characterizing a traditional Algerian cheese “Bouhezza”. Ph.D. Thesis in Food Science from the Institute of Nutrition, Food and Food Technology INATAA Constantine, Algeria. p. 27-107.
- Casalta, E. (2003). Scientific Bases of the Quality of the Venaco, traditional cheese from raw milk. Development of Specific Selected Ferments. Theses, University of Burgundy. Dijon, France.p.56-82.
- Delbes, C., Monnet, C., Irlinger, F. (2015). Microbial communities in the service of the quality of the cheeses. Agricultural Innovations. 44 (1): 69-86.
- Depouilly, A., Dufrene, F., Beuvier, E., Berthier, F. (2004). Genotypic British of the dynamics of the lactic acid bacterial population of County cheese, milk. 84(1): 155-167.
- Dubernet, S., Desmasures, N., Gueguen, M. (2008). Diversity and dynamics of lactobacilli populations during ripening of RDO Camembert cheese. Canadian Journal of Microbiology. 54(3): 218-228.
- Fricker, M., Skanseng, B., Rudi, K., Stassi, B., Ehling- Schulz, M. (2011). Shift from farm to dairy tank milk microbiota revealed by a polyphasic approach is independent from geographical origin. International Journal of Food Microbiology. 145(1): 24-30.
- Irlinger, F., Layec, S., Helinck, S., Dugat-Bony, E. (2015). Cheese rind microbial communities: diversity, composition and origin. FEMS Microbiol. Lett. 362(1-11): 10-19.

- Jadhav, B.S., Kulkarni, M.B., Chavan, K.D. (2008). Effect of lactation order and stage of lactation on physical properties of milk. *J. Dairying, Foods and H.S.* 27(3/4): 168-174.
- Jany, J.L. and Barber, G. (2008). Culture-independent methods for identifying microbial communities in cheese. *Food Microbiol.* 25(7): 839-848.
- Leclercq-Perlat, M.N. (2011). Cheese: Camembert, Brie and related varieties. In Editor in chief: John. WF, editor. *Encyclopedia of Dairy Sciences* (Second Edition). San Diego: Academic Press. pp. 773-782.
- Manzo, N., Santini, A., Pizzolongo, F., Aiello, A., Marrazzo, A., Meca, G., Durazzo, A., Lucarini, M., Romano, R. (2019). Influence of ripening on chemical characteristics of a traditional Italian cheese: Provolone del Monaco. *Sustainability.* 11(9): 2520-2528.
- Monnet, C., Landaud, S., Bonnarme, P., Swennen, D. (2015). Growth and adaptation of microorganisms on the cheese surface. *FEMS Microbiol. Lett.* 362(1): 1-9.
- Montel, M.C., Bouton, Y., Parguel, P. (2012). Ecosystems milks and cheeses milk issues for their mastery. *Renc. Rech. Ruminants, F - 15000 Aurillac. INRA.* 19(1): 545-560.
- O'Sullivan, D.J., Cotter, P.D., O'Sullivan, O., Goblin, L., Mcsweeney, P.L.H., Sheehan, J.J. (2015). Temporal and spatial differences in microbial composition during the manufacture of a continental-type cheese. *Applied and Environmental Microbiology.* 81(7) : 2525-2533.
- Quigley, L., O'Sullivan, O., Beresford, T.P., Ross, P., Fitzgerald, G.F., Cotter, P.D. (2011). Molecular approaches to analysis the microbial composition of raw milk and raw milk cheese. *International Journal of Food and Microbiology.* 150(2-3): 81-94.
- Reats, D., Offek, M., Minoz, D., Halpern, M. (2011). Molecular analysis of bacterial communities in raw cow milk and the impact of refrigeration on its structure and dynamics, *Food. Microb.* 28 (3): 465-471.
- Sakore, D.B., Dhole, P.T., Chavan, K.D., Pawar, B.K. (2007). Role and viability of probiotic cultures in cow milk DAHI. *J. Dairying, Foods and H.S.* 26(2): 63-68.
- Shekhar, C., Motina, E., Kumar, S. (2010). Microbiological quality of raw milk and its public health significance. *J. Dairying, Foods and H.S.* 29(1): 15-18.