

Assessment of the Functional Potential of Two Autochthonous Lactic Strains of Lactobacilli Isolated from Algerian Dairy **Products**

A.A Dahou¹, K. Doukani^{1,2}, H. Tahlaiti¹, H. Bouhenni², A. Bekihal¹, L. Doubbi-Bounouaa¹

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ABSTRACT

Background: In cheese-making technology, lactic coagulation of milk is an essential stage in which the use of a ferment may be a selected strain or a mixture of strains that do not all have the same performance and technological properties, resulting in a wide variation in the quality of the cheese product.

Methods: The aim of this study was to highlight the technological aptitudes of two strains of lactic acid bacteria, LAB, indigenous to our Algerian dairy products, Lactobacillus acidophilus and Lactobacillus brevis. The study monitored their growth kinetics, hydrogen ion concentration, fermentation profile and proteolytic activity on cheese milk.

Result: Both strains gave good results, with very good growth kinetics, a good hydrogen ion concentration for cheese processing and an excellent proteolytic profile (proteolytic activity at 1, 2 and 3%). These 02 LAB revealed good technological performance in terms of cheese application, for lactic fermentation, lactic coagulation and also through their noted proteolytic activity can lead to good cheese maturation during ripening. This assessment leads us to conclude that these strains could be used for cheese applications in single or mixed cultures and with the possibility of being used in a cheese processing technology with lactic dominance for fresh cheeses or mixed enzymatic and lactic dominance for soft and pressed cheeses.

Key words: Biotechnological aptitudes, Lactic coagulation, Lactic fermentation, Lactobacillus acidophilus, Lactobacillus brevis.

INTRODUCTION

The nomadic pastoralists of the steppe and sub-Saharan areas are the main consumers of local dairy products (Raib, Leben, farm butter of the Beldi type, traditional cheeses) throughout Algeria. This shows their attachment to their heritage, which is an integral part of their geography, history, culture and gastronomic tradition (Badis et al., 2005).

These local dairy products differ in shape, colour, texture, taste, etc. and are produced according to knowhow that differs from one region to another in contrast to the seasonal transhumance or diversified pastures (Dahou et al., 2015).

Traditional dairy products host a diverse microbiota, made up of endogenous microbial populations, which play a major role in the development of their nutritional and organoleptic qualities (Mechai et al., 2014). Scientific research has highlighted this diversity in dairy products by characterizing and understanding the role of the dairy microbial ecosystem in the processing of milk from various farms (Leroy and De Vuyst, 2004; Zhu et al., 2009; Montel et al., 2014). Initially, the aim was to better control the organoleptic quality of manufactured dairy derivatives by using flora with specific technological and aromatic functions. Subsequently, studies focused on the sanitary aspect. This research has led to a formidable technical evolution towards safe and regular dairy products. In recent years, research has focused on understanding the complex metabolic phenomena of the lactic flora that produce the dairy byproducts typical of each region. This diversity must be

¹Laboratory of Sciences and Techniques of Animal Production, Faculty of Nature and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria.

²Faculty of Nature and Life Sciences, Ibn Khaldoun University, Tiaret, Algeria.

Corresponding Author: A.A Dahou, Laboratory of Sciences and Techniques of Animal Production, Faculty of Nature and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria. Email: abdelkader.dahou@univ-mosta.dz

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considered as a heritage value. The latest innovation in food microbiology for the dairy industry is the adaptation of lactic bacteria cultures with controlled metabolic profiles to develop functional dairy products combining organoleptic quality and naturalness (Vignola and Amiot, 2002).

Among these LAB, lactobacilli are found in diverse environments, rich in carbohydrate substrates such as fermented or decomposing products. Some lactobacillus species are involved in the production of fermented food products such as fermented milks, lactic curds and ripened cheeses (Anjum et al., 2014; Saidane et al., 2021).

It is in this context that this study aims to evaluate the technological capabilities of two indigenous strains isolated from Algerian dairy products, *Lactobacillus acidophilus* and *Lactobacillus brevis*. This evaluation involved checking their purity, phenotypic re-characterization, monitoring their growth kinetics and studying their metabolic performance by assessing their fermentative profile and proteolytic activity. This whole approach is an initiative aimed at involving scientific research in the valorization of our indigenous lactic strains in local dairy processing.

MATERIALS AND METHODS

Site and period of study

Carrying out the study at the Research Laboratory of Sciences and Techniques of Animal Production, Experimental Farm, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University of Mostaganem, during the period from February 2022 to July 2022.

Animal material

The animal material is essentially skimmed, cheesable cow's milk, with a dry matter content of 12% and a protein content of 3.2%. This prepared milk showed no cell viability after double pasteurization, one in LTLT (Low Temperature Long Time) and a second in HTST (High Temperature Short Time) and refrigerated storage at 4°C.

The usefulness of double pasteurization is to avoid the viability of any microbial cells, as well as any enzymatic activity and thus any interaction with our tested LAB. The first moderate treatment (at 75°C with a 20-second heat treatment) destroyed all pathogenic microorganisms and reduced the total flora, with the presence of heat-resistant fungal flora. The second, complementary treatment (flash at 95°C with 5-second chambering) completely eliminated the persistent fungal flora in the experimental milk.

Biological material

The origin of the two lactic acid bacteria, *Lactobacillus acidophilus* and *Lactobacillus brevis*, is a previously established isolation from a local fresh cheese "J'ben" from the steppe regions of western Algeria. To confirm their purity, the 02 strains were phenotypically identified using the API galleries of bioMérieux in Marcy-l'Etoile, France, using the reference API 50 CHL version 5.2. In addition to 70% of skimmed milk, the strain preservation medium contained microbiological ingredients from the Institut Pasteur, Agiers, Algeria; composed of 0.05% yeast extract, 0.05% glucose and 30% glycerol.

Technological aptitude

Fermentation type

This test is used to differentiate between homofermentative and heterofermentative LAB. It consists in demonstrating the production of carbon dioxide CO₂. Gibson-Abdelmalek medium, a product of the Institut Pasteur, Algiers, Algeria, previously melted, cooled and solidified, was inoculated with the LAB strains studied, each in a tube by central pricking,

then a sterile agar plug was poured over the surface. Incubation took place at 37° C for 7 days. The development of a homofermentative bacterium does not lead to discontinuity between the medium and the agar plug. On the contrary, if metabolism is heterofermentative, induced CO_2 gas production pushes the agar plug up the tube (Badis *et al.*, 2005).

Study of growth kinetics of lactic strains by bacterial counting

Bacterial counting (bacterial growth) was carried out directly by a BactoScan FC FOSS, Denmark. This analysis complies with the IDF/ISO standard as the only rapid analysis equipment approved for counting bacterial cells in milk and milk derivatives (I.D.F, 2018). It was determined every 2 h for 24 h, on a volume of 100 ml of milk analyzed and expressed as the number of individual bacteria IBC/mL. Our results were expressed in millions of IBC/mL of milk analyzed (mn IBC/mL).

The BactoScan is a flow cytometry device, a technology that enables bacteria to be counted. The device sucks in 4.5 ml of milk and adds a dye to the sample, which stains the DNA of the bacteria, making them detectable by the device. If the bacteria are destroyed, their DNA will be free in the milk and will not be counted by the device. The milk sample is pumped into a very small tube illuminated by a laser, which detects lactic acid bacteria thanks to their fluorescence perceived by a highly sensitive sensor. This sensor sends out an electrical impulse each time a bacterium passes through. Each signal is counted, giving an individual bacterial count (IBC). This number is then converted per milliliter of milk to give the result noted (Morin, 2020).

Standardization of growth kinetics

This paramount control parameter is recommended by the International Dairy Federation (I.D.F, 2018), which tests the multiplication of lactic acid bacteria strains on sterile milk inoculated with 2-3% lactic sourdough for 24 h at 37°C. Lactic sourdough is composed of two separate pure cultures, one of Lactobacillus acidophilus and the other of Lactobacillus brevis, prepared on 100 ml of MRS (Man Rogosa Sharpe, from the manufacturer Dutscher France) and incubated at 37°C for a maximum of 24 h to reach a bacterial cell concentration exceeding 106 IBC/ml. This concentration is determined and obtained by assaying bacterial DNA with the Nano drop sample retention system spectrophotometer (Thermo Fisher Scientific Inc.). The principle of this spectrophotometry is based on the fact that nucleic acids present an absorption maximum in U.V. light, thus determining their concentration and purity (Quigley et al., 2011). DNA with a pure bacterial culture > at 106 IBC/ml gives a specific absorption peak at 260 nm.

Standardization of LAB growth kinetics is a necessary aggregation parameter in dairy processing that validates the concentration of bacterial cells of technological interest, which according to the IDF must reach a threshold > to 10⁶ IBC/mL after 6 h of incubation for thermophiles and after 18 h for mesophiles.

pH measurement

The pH parameter was measured directly, during the establishment of bacterial growth kinetics, using a HANNA Instruments INC, USA, pH meter specially designed for milk and milk derivatives, reference HI99162, by immersing the electrode in the volume of milk analyzed. The pH was determined every 2 hours for 24 hours (Salminen et al., 2004).

Proteolytic power

To determine the proteolytic activity of LAB, the lactobacillus-specific agar culture medium MRS (Man Rogosa Sharpe, from the manufacturer Dutscher France) was enriched with different doses of sterile skimmed milk (1%, 2% and 3%), solidified and dried in Petri dishes for this test (Kholif *et al.*, 2011). This medium was then inoculated with cultures of Lactobacillus acidophilus and Lactobacillus brevis using the multi-point plating technique. Inoculation was carried out on discs placed on the medium, each receiving a 20 μL volume of the bacterial culture under study. Plates were incubated at 37°C for 24 h. Proteolysis was revealed by clear areas around the discs.

Coagulant power

The principle of lactic coagulation is the change of state of milk from liquid to semi-solid, called gel or coagulum. The product then separates into two phases: whey and coagulum (Roudj *et al.*, 2009).

To achieve coagulant power, 100 mL of sterile skimmed milk was inoculated with 3 mL of each bacterial culture. After incubation at 37°C for 24 h, the volume of whey exuded was recorded and the appearance of the coagulum obtained was assessed with the naked eye.

Statistical analysis

The study of the similarity of the technological aptitudes of the native lactic strains studied in relation to the ATCC (American Type Culture Collection, 2022) reference strains, *Lactobacillus acidophilus* ATCC 314 and *Lactobacillus brevis* ATCC 14869, was determined using a statistical test in SYSTAT MYSTAT 13 SOFTWARE. The level of statistical significance was determined as follows: Differences were considered non significant when P<0.05. P>0.05 significant difference.

RESULTS AND DISCUSSION

Technological aptitude results

Investigation of fermentation type

Our 02 strains studied (Fig 1) conformed to the LAB growth standard and to the standard defined for the ATCC reference strains: Lactobacillus acidophilus ATCC 314 and Lactobacillus brevis ATCC 14869. This test confirmed that Lactobacillus acidophilus was homofermentative (Fig 2), as demonstrated by the absence of CO₂ production in tube A (no agar detachment). Lactobacillus brevis, on the other

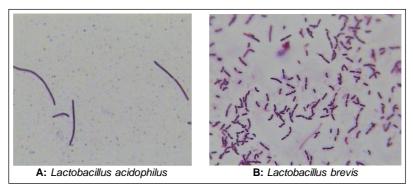


Fig 1: Microscopic aspect and arrangement of lactic strains, after Gram staining (M × 100).

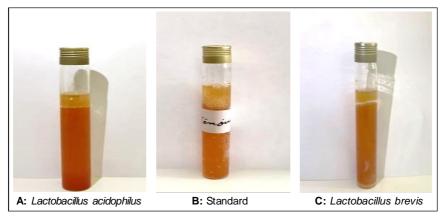


Fig 2: Fermentation type of the lactic strains studied after 7 days of incubation at 37°C.

hand, was heterofermentative, as evidenced by the production of CO_2 in tube C. There was agar detachment, while tube A remained identical to tube B (control). The development of a homofermentative bacterium did not cause any detachment between the Gibson-Abdelmalek culture agars and the top agar. The gas produced by heterofermentative metabolism pushes the agar plug towards the top of the tube (Ketrouci *et al.*, 2021 and Sadi *et al.*, 2017).

Results of lactic acid bacterial growth kinetics

Following the evolution of bacterial numbers and pH as a function of time, the results obtained are shown in Fig 3 and 4.

The notion of bacterial growth norm covers two aspects: bacterial cell growth (size, mass, volume) and the phenomenon of cell division (population). In addition, this

growth induces a series of metabolic reactions leading to the production of cellular biomass. Growth duration was normative (visible in figures 3 and 4 in mn IBC/ ml), > to 6 h for the thermophilic strain with a peak in bacterial cell multiplication at 12 h and \leq to 18 h for the mesophilic strain. These kinetics lead to the production of a lactic curd, visibly defined by the increase in lactic biomass in the lactic coagulum produced and the controlled syneresis of whey.

pH measurement

Fig 5 shows the evolution of the hydrogen ion pH concentration of the *Lactobacillus acidophilus* and *Lactobacillus brevis* lactic strains studied as a function of time (h) during growth kinetics measurements.

Kholif et al. (2011) obtained similar results with lactobacilli. Lactic acid, derived from the breakdown of

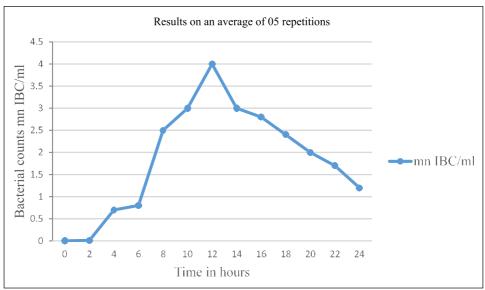


Fig 3: Evolution of the bacterial count of the pure culture of Lactobacillus acidophilus.

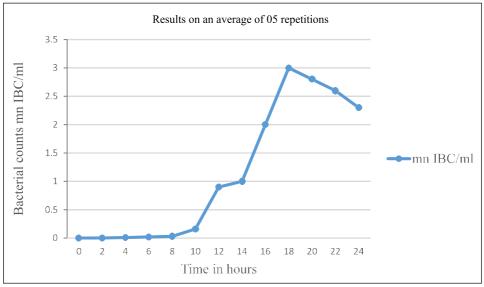


Fig 4: Evolution of the bacterial count of the pure culture of Lactobacillus brevis.

lactose by lactic acid bacteria, carries positive charges (hydrogen ions) which neutralize the negative charges of colloids (caseins). At pH 4.6-4.8, known as casein's isoelectric point, they become neutral. The acid thus dehydrates the casein micelles, bringing them closer together. The greater the demineralization (acidification of the milk by lactic bacteria or acid coagulation), the more lactic the curd.

According to our results and Fig 5, at 0 h, the initial pH of cheese milk inoculated with two cultures of *Lactobacillus acidophilus* and *Lactobacillus brevis* strains is close to neutral, with an average of 6.67. Isoelectric pH (around 4.6 - 4.8) with the *Lactobacillus acidophilus* strain was reached after more than 12 h of cheese milk inoculation, while for *Lactobacillus brevis*, isoelectric pH was reached after more than 17 h of incubation. For both strains studied, cheese milk underwent controlled post-acidification even after 24 h incubation at a pH below 4 for 02 LAB.

Indeed, lowering the pH plays an essential role, on the one hand in the lactic coagulation of the milk by destabilizing the casein micelles that lead to the formation of a homogeneous lactic gel and on the other hand, by conferring

bio-protection and a distinctive taste to the milk derivative that contributes to its flavor and aroma (Leroy and De Vuyst, 2004; Mechai *et al.*, 2014 and Saidane *et al.*, 2021).

Proteolytic power

The proteolytic systems of LAB are important in the curd ripening processes of manufactured cheeses, which give cheeses their rheological properties and organoleptic characteristics (Kholif *et al.*, 2011 and Roudj *et al.*, 2009). On the other hand, the proteolytic activity of LAB is essential for their growth in milk, as well as for the development of organic metabolites essential for the bioprotection of milk-derived products.

The result of the proteolysis test is a lysis halo around the bacterial colony, the diameter of which is measured to assess the intensity of proteolytic activity (Salminen *et al.*, 2004). The results showed that *Lactobacillus acidophilus* was highly proteolytic compared to *Lactobacillus brevis* (Fig 6), the same results obtained by Saidane *et al.* (2021), who showed that in similar media, proteolytic activity is induced by both mesophilic and thermophilic lactobacilli.

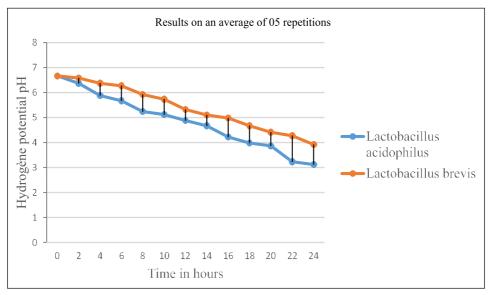


Fig 5: Average pH kinetics of Lactobacillus acidophilus and Lactobacillus brevis cultures.

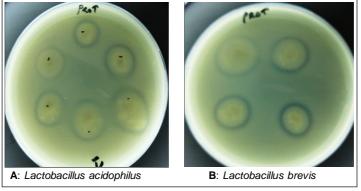


Fig 6: Proteolytic activity of Lactobacillus acidophilus and Lactobacillus brevis.

According to the table, proteolytic activity was variable from one dose to the next, with the best proteolysis zone obtained at the 1% dose in *Lactobacillus acidophilus*, with a high level of proteolysis reflected in a lysis diameter of 7.25 mm and at the 2% dose, the strain showed average proteolysis with a diameter of 2.1 mm. At the 3% dose, the strain was weakly proteolytic, with a lysis zone 1.3 mm in diameter. On the other hand, *Lactobacillus brevis* has very low proteolytic activity, as shown in Table 1. At a dose of 1%, proteolytic activity was minimal at 0.5 mm diameter. At a dose of 2%, proteolytic activity was triggered with a lysis zone of 0.98 mm in diameter. At a dose of 3%, the culture began to show proteolytic activity with an average of 1.86 mm in diameter, but this was still minimal compared with *Lactobacillus acidophilus*.

According to Anjum et al. (2014) and Sadi et al. (2017), a lactic strain can be confirmed as proteolytic if it develops a lysis zone characterized by a halo around the bacterial colony with a diameter of between 5 and 15 mm. If we compare these data with our results, we can say that Lactobacillus acidophilus has a more proteolytic profile than Lactobacillus brevis.

Coagulant power

Lactic coagulation determined after a 24-hour incubation period; milk prepared and inoculated with a mixed culture of the two lactobacilli, one mesophilic and the other thermophilic.

Total lactic coagulation was triggered at an isoelectric pH of 4.8, where the negative charges of kappa caseins are neutralized by H+ ions from lactic fermentation. The acid dehydrated the casein micelles, leading to their assembly by strong, irreversible bonds.

According to Leroy and De Vuyst, (2004) and Vignola and Amiot, (2002), the concentration of hydrogen ions is decisive for good lactic coagulation, at the isoelectric pH of milk (pH between 4.6 and 4.8): caseins (insoluble milk proteins) lose their negative charge and bind together by weak bonds. They create a mesh that solidifies the milk, giving it a "thick" consistency. This is how lactic curd is obtained from lactic coagulation.

Roudj et al. (2009), acknowledges that this coagulation method depends on the dose of bacterial culture and the type of LAB used, the quality of the milk and its incubation temperature during acidification. Several hours to dozens of hours are required to obtain satisfactory curdling. Vignola and Amiot, (2002) recommend 04 to 06 hours for thermophilic lactic ferments and 12 to 18 hours for mesophilic ferments.

Our results are also in line with IDF standards for cheese processing. Through their metabolic activities, the strains studied induced a hydrogen ion concentration with the coagulation activities sought in cheese applications, adaptable to either lactic or mixed curd type cheeses. The coagulates obtained for both strains were perceived by the naked eye as firm, non-friable, with a shiny, non-degraded gel. Non-abundant post-acidification resulted in low whey exudation with a > content of 20% for an IDF-defined whey exudation standard of 15-30%. This explains why any successful dairy derivative requires the right choice of lactic acid bacteria used, substrate control and adaptation of technological parameters to obtain typical dairy products highly appreciated by the consumer.

Statistical analysis

The technological performance of autochthonous lactic acid bacteria isolated from a J'ben terroir cheese with lactic curd showed a similarity in the growth kinetics and acidification profiles studied, demonstrating a good metabolic profile with a controlled rise in hydrogen ions giving isoelectric pHs in line with a lactic coagulation that is both mesophilic and thermophilic. This comparison was made against the data sheets for ATCC reference strains of the same LAB species, Lactobacillus acidophilus and Lactobacillus brevis. These certified data sheets determine the performance of lactic acid bacteria in terms of their fermentative profile (acidifying power-lactic acid production) and proteolytic activities for typical dairy processing. Proteolysis by hydrolysis of skim milk proteins varied significantly (P>0.05) according to the doses used and the bacterial culture tested. Taking into account the same research established in the context of LAB proteolytic activities, including those of Dahou et al. (2015); Roudj et al. (2009); Salminen et al. (2004); Zhu et al, 2009) and our results obtained with proteolysis evolving

Table 1: Average proteolytic diameter in millimetres for the strains studied after 24 h of incubation.

Repetitions	L1			L2		
	D1	D2	D3	D1	D2	D3
1	5.0	2.4	1.2	0.7	2.2	2.1
2	6.5	2.2	1.5	0.6	0.9	2.3
3	7.25	1.8	1.2	0.7	1.0	1.3
4	8.5	2.1	1.5	0.5	-	2.0
5	9.0	2.0	1.1	-	0.8	1.6
Average in mm	7.25*	2.1*	1.3*	0.5*	0.98*	1.86*

L1: Lactobacillus acidophilus; L2: Lactobacillus brevis; D1: 1% skimmed milk dose; D2: 2% skimmed milk dose; D3: 3% skimmed milk dose; -: Negative test.

^{*}The mean proteolytic activity, at different doses of skimmed milk, of the 02 LAB cultures showed a significant difference P>0.05.

with the protein content of the milk used. This proves that our native cultures are adaptable to low and high protein milks, as is the case with our local milks, which show a fluctuation in protein content that weakens as the lactation phases of the dairy herd evolve.

According to the results obtained, these LABs from the Algerian dairy terroir adapt well to the availability and qualitative typicity of local milks for the production of a variety of highly consumed fermented dairy and cheese products, which represent an Algerian heritage of great dietary, medicinal and economic importance.

CONCLUSION

Evaluation of autochthonous lactic strains isolated from dairy products from western Algeria confirmed the typicality of two lactobacillus species: Lactobacillus acidophilus and Lactobacillus brevis. The functional potential of the 02 lactobacilli through their technological aptitudes was in line with the reference strains presenting a fermentative acidophilic profile with proteolytic activity favoring the production of growth factors and amino acids necessary for the development of highly dependent secondary lactic microflora.

Furthermore, the prospects of this study will be, in more ways than one, convincing by working on a better knowledge of the dairy microbial ecosystems of our local products and by proposing, with the help of our research teams, the use of cocktails of selected strains, with the capacity to reproduce metabolisms of interest in dairy processing and giving originality to the products manufactured. What's more, with these technological assessments of our indigenous lactic flora, we're beginning to get a reliable picture of the microbial diversity of our traditional dairy products, which we'll need to exploit further to enhance their value and preserve their typicality and work towards a protected designation of origin for the heritage of dairy derivatives.

Conflict of interest

All authors declared that there is no conflict of interest.

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