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Isolation and Characterization of Lactic Acid Bacteria in Traditional Algerian Smen

Hassina Guetarni[1,2]*, Yassine Hennine[1] and Houcine Seriè[1]

[1]*Department of Biology, Faculty of Nature and Life Sciences and Earth Sciences, Bounaama Djilali University of Khemis Miliana, 44225, Ain Defla, Algeria*

[2]*Natural Substances Valorization Laboratory, Bounaama Djilali of Khemis Miliana University, 44225, Ain Defla, Algeria*

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Abstract: Like other fermented dairy products, smen (a kind of traditional salted and fermented butter in Algeria) provides a favorable ecological niche for lactic acid bacteria (LAB). This study aimed to isolate, characterize, and identify LAB present in traditionally prepared Algerian smen. Three smen samples were collected from different regions of the Wilaya of Ain Defla and subjected to phenotypic, biochemical, and physiological analyses. A total of twenty LAB isolates were recovered, belonging to three genera: *Enterococcus*, *Pediococcus*, and *Lactococcus*. The genus *Enterococcus* was predominant, representing 60% of the isolates, likely due to its strong adaptability to the harsh conditions associated with smen production, including heating, salting, and anaerobic storage. The identified species included *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus malodoratus*, *Enterococcus dispar*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactococcus lactis* subsp. *lactis*, and *Lactococcus garvieae*. The isolates were further evaluated for key biotechnological traits, with emphasis on acidification capacity and lipolytic activity, both of which are critical to product quality and flavor development. The scientific validation of traditional products such as smen not only supports their safety and functional potential but also facilitates their transformation into standardized, value-added foods. This research contributes to promoting local agro-food expertise and enhancing market opportunities for producers in arid regions.

Key words: Milk, smen, lactic acid bacteria, *Enterococcus*, *Lactococcus*, *Pediococcus*.

Correspondence
Hassina Guetarni
kmhg2009@yahoo.fr

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Milk is a fragile product that is highly alterable microbially due to its high-water content, near neutral pH and its richness in biodegradable components (lactose, proteins and lipids). To increase its shelf life, man since antiquity has worked on its fermentation and transformation into other better preserved dairy products. The fermentation of milk has been used for a very long time to prolong its preservation. Indeed, all human populations practicing livestock farming have been able to develop traditional methods of fermenting the milk of their herds (cows or others) to produce fermented

dairy products, some of which are now also manufactured industrially (Yantyati and Andi, 2014). In Algeria, traditional dairy products are produced using indigenous manufacturing methods rooted in the cultural heritage of local communities and transmitted across generations. These products include *lbene*, *raib*, *smen*, *jben*, *bouhezza*, *klila*, among others (Lahsaoui, 2009). In addition to being easier to store, these products are also more digestible than original milk and their organoleptic qualities are highly valued. Smen is a traditional dairy product (a kind of traditional salted and fermented butter in Algeria) and an essential ingredient in Algerian cuisine, and widely known also in other North African and Middle Eastern countries. It is an aggregate of fat of animal origin (farm butter) which has subsequently been washed, salted and kneaded and then packaged in hermetically closed jars and stored in a cool, dark place at room temperature (El Marrakchi *et al.*, 1986; Sakili and Issouel, 2003). This product, highly appreciated by the Algerian consumer for its taste and dietary qualities, is used as an additive for food products in order to enhance the taste and aroma of certain traditional Algerian recipes (couscous, tagine, rfisse, assida, etc.). Its property as a high-energy food is exploited in traditional medicine to alleviate the pain caused by the sensation of cold which accompanies cough, rheumatism and bone trauma (oral and massage) (Sakili and Issouel, 2003).

Despite its high consumption, its availability remains low. This is due to the absence of industries dedicated to the development of this product and insufficient milk production due to the lack of breeding. Indeed, Algeria ranks second in the world in terms of imports of milk and dairy products after Mexico, and milk represents 20% of its total food imports (Souki, 2009). This leads the Algerian population to consume products of plant origin (vegetable smen and margarine). Among all microorganisms, lactic acid bacteria play a major role in the fermentation of milk, and contribute to the refining and maturation of several fermented dairy products. As a result, they also have a great interest in the food industries where they are widely used in the fermentation of several animal and vegetable raw materials and they are also known for their beneficial effects on health. Their use as a probiotic contributes to the improvement of digestive functions and the healing of the imbalance of the gastrointestinal microflora, and helps in the maturation of the immune system. The most well-known genera of lactic acid bacteria used in the food industries are: *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Bifidobacterium* (Leveau and Bouix, 1993). Due to the important place occupied by lactic acid bacteria in the fermentation and production of fermented dairy products and their refining, we thought of determining the main agents in the transformation and maturation of farm butter into smen, and the improvement of its taste and organoleptic characteristics. This study aims to isolate and identify the lactic acid bacteria (LAB) within traditional Algerian smen and also evaluating their acidifying and lipolytic potential. By establishing this biotechnological profile, the research aims to provides the necessary groundwork to transition of artisanal expertise into standardized, industrial-scale functional foods.

Materials and Methods

Three smen samples were used in this study. They were taken from different regions of the wilaya of Ain defla (one sample from each location). Bourached (36° 10′ 9″ North, 1° 55′ 45″ East), Ain Lechiakh (36° 09′ 25″ North, 2° 24′ 15″ East) and Ben Allal (36.3123° North, 2.1652° East). These samples represented varying maturation periods, with fermentation times of over four months, ten years, and six months, respectively. The exceptionally long preservation period observed in the Ain Lechiakh sample (+10 years) reflects a traditional Algerian practice intended to significantly enhance the product's characteristic organoleptic qualities through extended aging.

Isolation of lactic acid bacteria

Preparation of stock solution and decimal dilutions: In order to prepare the stock solution, 2.6 g of smen sample contained in a sterile tube was added with 2 mL of physiological water (sterile 0.9% sodium chloride solution in purified water), then incubated at 37°C until merging and obtaining two phases. Then, to ensure the distribution of the bacteria, agitation for 3 to 5 minutes using a vortex was carried out (Sakili and Issouel, 2003). Decimal dilutions up to 10^{-6} were prepared from the stock solution: using a

sterile Pasteur pipette, a volume of 1 mL of the aqueous phase was taken and added aseptically to 9 mL of physiological water to obtain of the 10^{-1} dilution and the process was continued like this until the last dilution was obtained.

Seeding, isolation and purification of lactic isolates: Seeding was carried out using three decimal dilutions: 10^{-3}, 10^{-4} and 10^{-5} by the rake spreading technique using a Pasteur pipette. Each dilution was inoculated on two MRS (De Man-Rogosa-Sharpe) and M17 (Terzaghi and Sandine) agar media, previously poured into sterile Petri dishes. These boxes were tightly closed to ensure anaerobiosis which is favourable for the development of lactic acid bacteria. Then, they were incubated at 37°C for 48 h to 72 h. After incubation, the colonies obtained were selected after visual macroscopic analysis (Guetarni *et al.,* 2012), Gram staining and catalase test.

The catalase test was performed by placing a drop of hydrogen peroxide onto a sterile glass slide, followed by mixing a colony of the test isolate into the reagent. The isolate was considered catalase-positive if immediate effervescence (bubble formation) occurred, indicating the presence of catalase enzyme activity. The absence of bubble formation indicated a catalase-negative reaction (Guetarni *et al.,* 2024). Gram staining was performed to differentiate Gram-positive from Gram-negative bacteria and to determine cell morphology and cellular arrangement. Heat-fixed bacterial smears on glass slides were flooded with gentian violet and allowed to stand for 1 minute. The slides were then rinsed gently with tap water and treated with Lugol's iodine for 1 minute to fix the primary stain. Decolorization was carried out using 95% ethanol for 30 seconds, followed by rinsing with water. The slides were subsequently counterstained with Ziehl fuchsin for 30 seconds. After a final rinse, the slides were air-dried and examined under a light microscope using oil immersion (Larpent and Larpent, 1990). Bacteria that retained the violet stain and appeared purple were recorded as Gram-positive, whereas bacteria that lost the primary stain and appeared pink or red after counterstaining were classified as Gram-negative.

Only those which present a typical appearance of lactic acid bacteria colonies, and which have Gram +ve and catalase -ve, were presumed to be lactic acid bacteria, and were inoculated in tubes containing MRS broth for their enrichment. Several subcultures were carried out for each isolated until colonies of the same size, same shape and same colour were obtained.

Biochemical and physiological identification of lactic acid bacteria

Oxidase test: It consists of taking part of a pure colony of MRS or M17 agar medium and placing it in contact with an "Ox" disk. The development of a purple colour means that the test is positive and that the isolate has the enzyme cytochrome oxidase (Kovacs *et al.,* 1995; Guetarni and Kararmouch, 2023).

Mannitol mobility: This test makes it possible to study the fermentation of mannitol and the mobility of bacteria. The isolates were seeded by central puncture to the bottom of the agar using a Pasteur pipette. Fermentation of mannitol results in colour change of the medium from red to yellow. Mobile bacteria move from the seeding line creating a disturbance in the medium, while immobile bacteria grow only along the seeding line (Gerhardt *et al.,* 1994).

Fermentative type: This test makes it possible to differentiate between homofermentative and heterofermentative lactic acid bacteria (production of CO_2 gas). The isolates were inoculated in tubes each containing 10 mL of BCP + L (Bromocresol Purple + Lactose) medium and a Durham bell. After incubation at 37°C for 24 to 48 hours, the CO_2 produced by the heterofermentative bacteria accumulates in the Durham bells (Copolla *et al.,* 1997).

Resistance to tellurite: Resistance to tellurite was investigated using MRS agar supplemented with 0.4% tellurite. The isolates were streaked on the surface of the medium, then incubated at 37°C for 24 h. Those which give black colonies after incubation are considered resistant to tellurite (Facklam, 1972).

Culturing at different temperatures: This culture makes it possible to distinguish between mesophilic and thermophilic lactic acid bacteria. After inoculation in MRS broth, the isolated were incubated at 15°C and 45°C for 24 h to 48 h (Guiraud, 2003).

Growth in hostile conditions: Bacteria growth under hostile conditions was assessed using four different tests. In first MRS broth was added with 2%, 4% and 6.5% NaCl, which was then distributed into tubes and inoculated with the isolated lactic acid producing bacteria. The result obtained after incubation at 37°C for 24 hours to 48 hours is considered positive if turbidity appears (Badis *et al*., 2005). In second the pH of the MRS medium was adjusted to 4.5, 6.5 and 9.5 using an HCl solution and then it was inoculated with the different isolates and incubated at 37°C for 2 to 3 days. The presence of cloudiness indicates bacterial growth (Badis *et al.,* 2005). Subsequently heat resistance was assessed. For this the tubes containing the MRS medium and inoculated with the isolates were incubated at 65°C for 30 minutes, after which they were cooled and incubated at 37°C for 24 hours. The presence of cloudiness indicates bacterial growth and that the isolate is heat-resistant (Guiraud, 2003).

Biochemical Identification using API 20E System: Although typically designed for *Enterobacteriaceae*, the API 20E gallery (bioMérieux, France) was utilized to assess specific biochemical markers and complement the identification of the isolates (Guetarni and Labdi, 2022; Guetarni *et al*., 2023). Key tests included arginine dihydrolase (ADH), citrate utilization (CIT), the Voges-Proskauer reaction (VP), gelatin liquefaction (GEL), and nitrate reduction (NIT). Additionally, the fermentation of several carbohydrates was evaluated, including glucose (GLU), mannose (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY), and arabinose (ARA).

The galleries were prepared by creating a humid chamber with sterile distilled water. For the inoculum, 2 to 3 colonies were suspended in 5 mL of sterile physiological saline to achieve high turbidity. Each microtube was inoculated via a sterile Pasteur pipette, ensuring the CIT, VP, and GEL cupules were fully filled, while the ADH test was sealed with sterile paraffin oil to maintain anaerobic conditions. The galleries were incubated at 37°C for 24 hours. Results were interpreted through direct colorimetric observation or following the addition of specific reagents (VP1/VP2 and Nitrite 1/Nitrite 2).

Hemolytic activity: To evaluate the safety profile and hemolytic character of the isolates, strains were cultured on MRS and M17 media supplemented with human blood and incubated at 37°C for 24 hours (Idoui *et al*., 2009). Hemolysis was classified as α-hemolytic (partial hemolysis, green zone), β-hemolytic (total hemolysis, clear zone), or γ-hemolytic (no change in medium).

Carbohydrate fermentation profile: The fermentative capacity of the isolates was determined using Bromocresol Purple (BCP) broth supplemented with 1% (w/v) of specific sugars, including lactose, fructose, maltose, and galactose, in addition to those present in the API gallery. Inoculated tubes were sealed with a layer of sterile petroleum jelly to ensure anaerobiosis and incubated at 37°C for 24 to 48 hours. A positive result was recorded upon the observation of microbial turbidity and a color shift from purple to yellow, indicating acid production.

Study of some technological properties of lactic acid bacteria

Acidifying power: It consists of studying on the one hand the evolution of the pH over time of the different cultures of skimmed milk where the isolates are cultivated. And to simultaneously study Dornic acidity by dosing with soda, and using the colored Phenolphthalein indicator which goes from colorless to pink. The analysis of these two parameters was carried out for each of the lactic acid isolated at different time intervals: 0 h, 2 h, 4 h, 6 h, 8 h and 24 h (Larpent, 1997).

Lipolytic power: Lipolytic activity was tested on MRS medium buffered at pH = 7 and supplemented with 30% Tween 80 (Guiraud and Galzy, 1980). Each isolate of a fresh culture was loaded into a Wattman disk (6 mm) then placed on the surface of the medium. Incubation lasted for 7 days at 37°C. Lipolytic activity results in the appearance of an opaque halo around the colonies (Guiraud and Galzy, 1980).

Results and Discussion

A total of 20 lactic acid bacteria (LAB) isolates were recovered from three traditionally produced smen samples (eight from Sample 1, six from Sample 2, and six from Sample 3). Their biochemical and physiological profiles are presented in Table 1 and 2.

Table 1. Results of phenotypic, biochemical and physiological tests of lactic acid isolated from the three smen samples

Isolates	Catalase	Gram	Oxydase	Fermentative type	Growth at different NaCl concentrations			Growth at different temperatures		Growth at different temperatures			Thermoresistance	Hemolysis test	Man Mob		Polassium tellurite	nitrate reductase	ADH	CIT	VP	GEL
					2%	4%	6.5%	15°C	45°C	pH 4.5	pH 6.5	pH 9.5			Mob	Man						
Sample 1																						
1	-	+	-	Hom	+	+	-	-	+	-	+	+	+	ɣ	-	+	+	-	+	-	-	-
2	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	-	-	+	-	+/-	-
3	-	+	-	Hom	+	+	+	-	-	-	+	+	+	ɣ	-	+	-	-	+	-	+	-
4	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	+	-	+	-	+	-
5	-	+	-	Hom	+	+	-	-	-	+	+	+	+	ɣ	-	+	-	-	+	-	+	-
6	-	+	-	Hom	+	+	+	-	+	+/-	+	+	+	ɣ	-	+	-	-	+	-	+/-	-
7	-	+	-	Hom	+	+	+	+	+	+/-	+	+	+	ɣ	-	+	-	-	+	-	+	-
8	-	+	-	Hom	+	+	+	+	+	+/-	+	+	+	ɣ	-	+	-	-	+	-	+	-
Sample 2																						
9	-	+	-	Hom	+	+	+	-	+	-	+	+	+	ɣ	-	+	+	-	+	-	+	-
10	-	+	-	Hom	+	-	-	-	+	-	+	+	+	ɣ	-	+	-	-	+	-	-	-
11	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	-	-	+	-	+	-
12	-	+	-	Hom	+	+	+	-	+	+/-	+	+	+	ɣ	-	+	+	-	+	-	-	-
13	-	+	-	Hom	+	+	-	-	+	+	+	+	+	ɣ	-	+	-	-	+	-	+	-
14	-	+	-	Hom	+	+	+	-	+	-	+	+	+	ɣ	-	+	+	-	+	-	-	-
Sample 3																						
15	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	+	-	+	-	+	-
16	-	+	-	Hom	+	+	+	-	-	+	+	+	+	ɣ	-	+	+	-	+	-	-	-
17	-	+	-	Hom	+	+	-	-	+	-	+	+	+	ɣ	-	+	-	-	+	-	-	-
18	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	+	-	+	-	+	-
19	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	-	-	+	-	-	-
20	-	+	-	Hom	+	+	+	-	+	+	+	+	+	ɣ	-	+	+	-	+	-	+	-

Man Mob: Mannitol Mobility; Mob: Mobility; Man: Mannitol; ADH: Arginine dihydrolase; CIT: Citrate; VP: Voges-Proskauer; GEL: Gelatin.

Carbohydrate utilization and metabolic diversity: All isolates metabolized primary dairy-associated carbohydrates (glucose, lactose, galactose, fructose, maltose, and sucrose), ensuring robust fermentation performance (Table 2). However, variability in the utilization of secondary sugars revealed strain-level metabolic heterogeneity, underscoring the functional diversity within the LAB consortium. This heterogeneity may contribute to differential acidification kinetics and flavor precursor formation, thereby influencing the sensory complexity of smen.

Colonies selected on MRS and M17 agar were morphologically consistent, appearing round, smooth, whitish, and slightly convex, with stable phenotypes across repeated subculturing. Microscopic observation confirmed that all isolates were Gram-positive cocci, exhibiting strain-dependent cellular arrangements including single cells, diplococci, short chains, tetrads, and clusters. Diplococci and short chains predominated, a pattern characteristic of lactic cocci adapted to dairy matrices.

All retained isolates were catalase- and oxidase-negative, non-motile, and capable of fermenting mannitol, confirming their classification as lactic acid bacteria (Table 1). None produced gas from lactose, indicating a strictly homofermentative metabolic pathway, consistent with efficient lactic acid production rather than mixed-acid fermentation (Table 2).

Stress tolerance and physiological adaptation

A defining feature of the recovered LAB community was its marked tolerance to thermal, osmotic, and pH stress. The majority of isolates

Table 2. Results of the fermentation profile of the isolated from the three samples

Isolates	Glucose	Galactose	Fructose	Saccharose	Maltose	Mannitol	Inositol	Sorbitol	Rhamnose	Melibiose	Amygladine	Arabinose	Lactose
Sample 1													
1	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	-	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+/-	+
8	+	+	+	+	+	+	+	+	+	+/-	+/-	-	+
Sample 2													
9	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	-	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	-	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+/-	+
13	+	+	-	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+/-	+
Sample 3													
15	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+

grew at 45°C, and all survived heat exposure at 65°C for 30 minutes, reflecting adaptation to the high-temperature conditions imposed during smen cooking. Furthermore, most isolates tolerated a broad pH range (4.5-9.5) and NaCl concentrations up to 6.5%, indicating strong ecological fitness in salted and aged butter environments.

All strains were arginine dihydrolase positive, suggesting an enhanced capacity for energy generation and pH homeostasis under nutrient- and acid-stressed conditions. In contrast, none utilized citrate, reduced nitrate, or liquefied gelatin, reinforcing their identity as specialized dairy-associated LAB. The absence of hemolytic activity further supports their low virulence potential, an important consideration for food safety.

Taxonomic Structure and Dominance Patterns

Overall phenotypic and biochemical profiling assigned isolates to three LAB genera: *Enterococcus* - 60% (dominant); *Pediococcus* - 25% and *Lactococcus* - 15%.

Species-level identification revealed the presence of *Enterococcus faecium*, *E. faecalis*, *E. malodoratus*, *E. dispar*, *Pediococcus acidilactici*, *P. pentosaceus*, *Lactococcus lactis* subsp. *lactis*, and *Lactococcus garvieae*.

The strong predominance of *Enterococcus* likely reflects its superior capacity to withstand heat, salinity, oxidative stress, and nutrient limitation, traits that confer a competitive advantage under the harsh physicochemical conditions of smen maturation. These findings position *Enterococcus* as a core adaptive taxon in Algerian smen, potentially shaping both

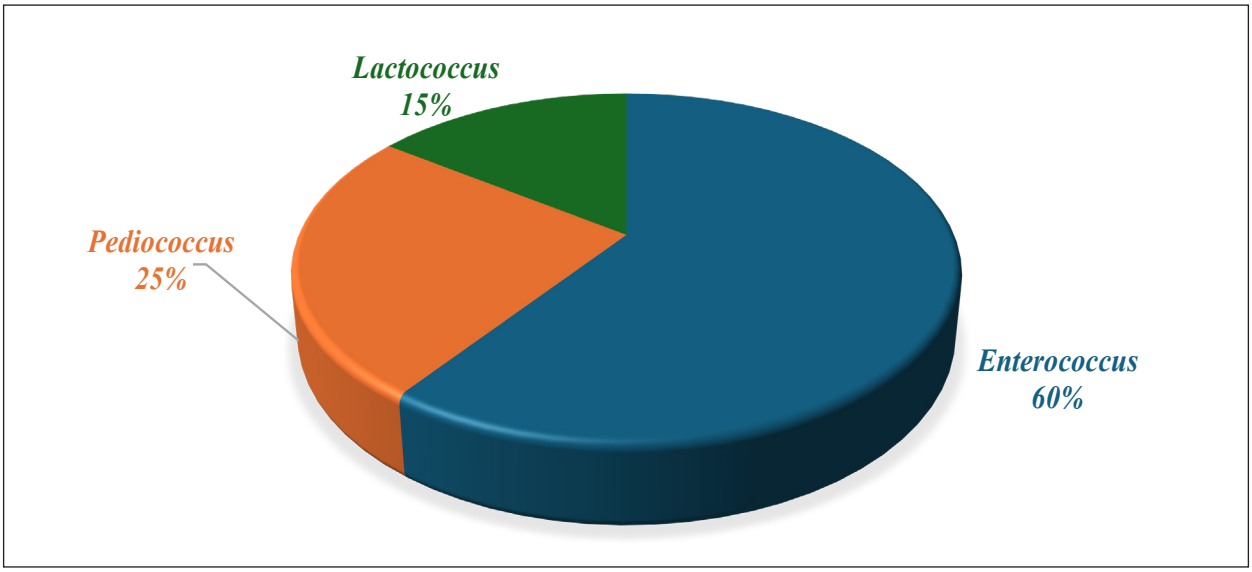


Fig. 1. Percentage of genera obtained and isolated from the three smen samples.

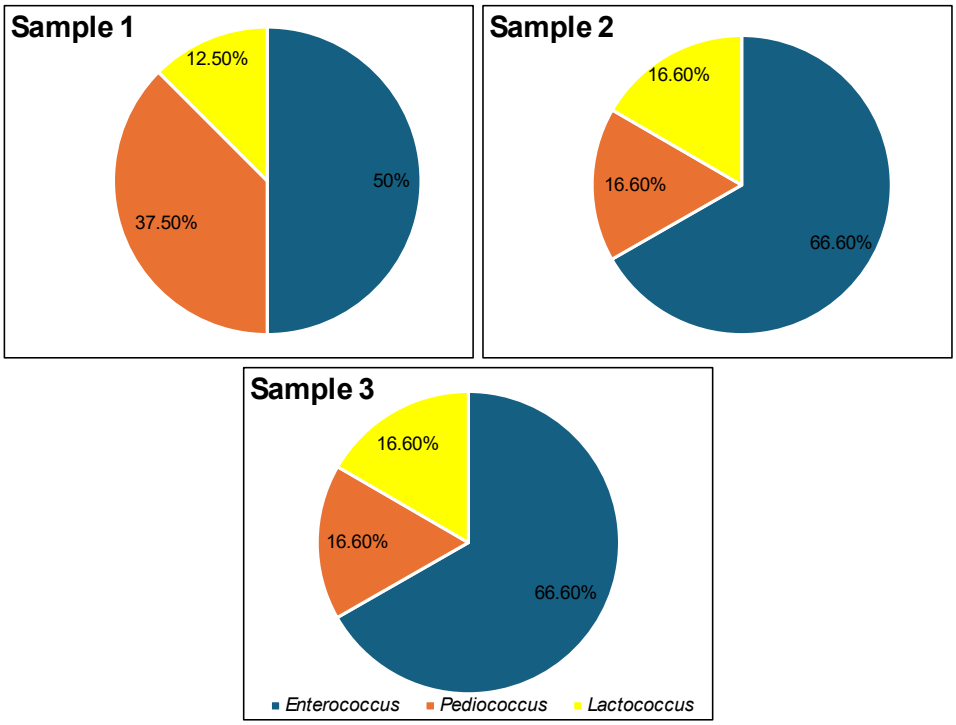


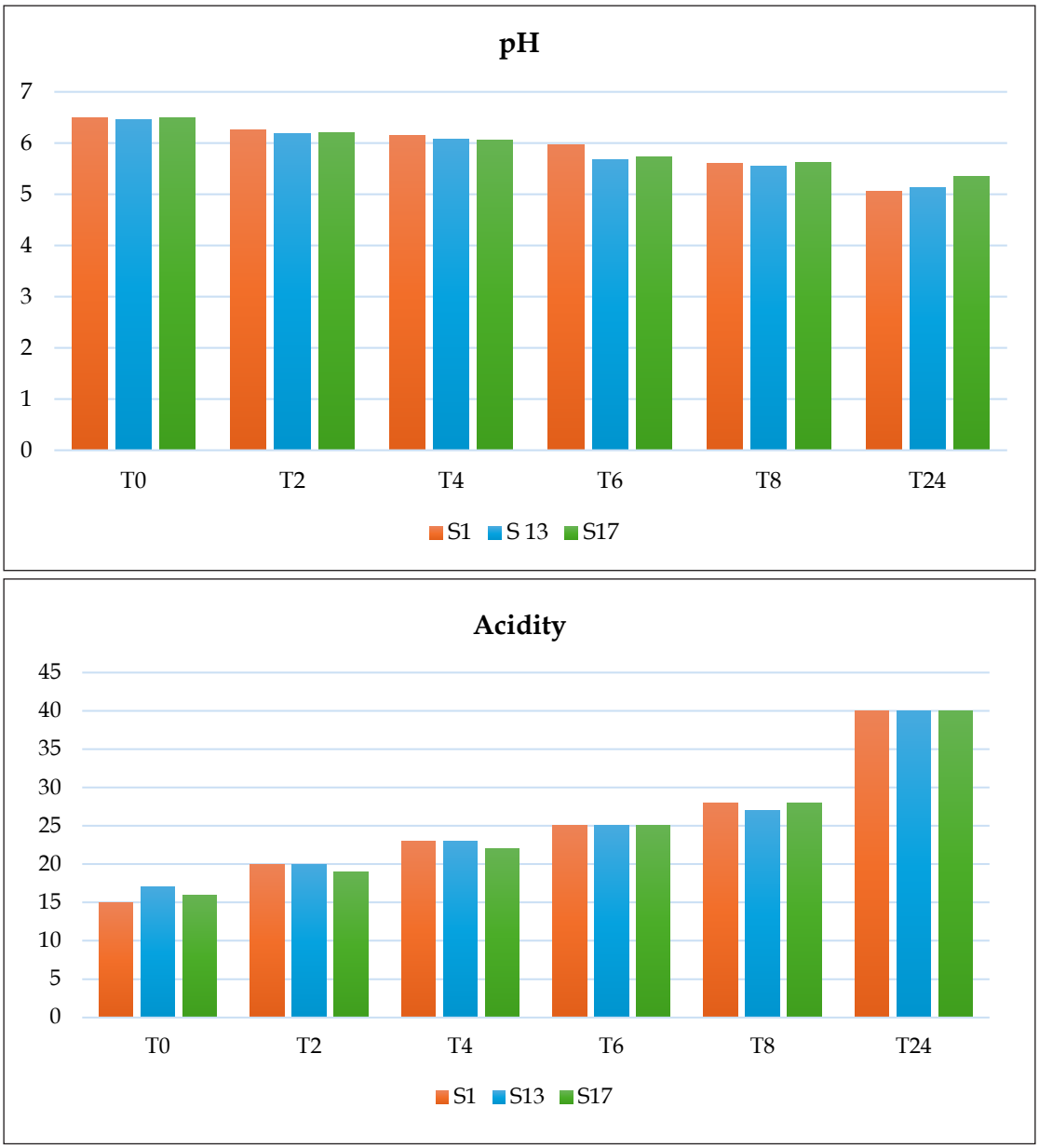
Fig. 2. Distribution of the three genera of lactic acid bacteria isolated in each smen sample analyzed.

microbial succession and biochemical evolution during ripening.

Technological performance and functional implications

Acidification Dynamics: All isolates exhibited progressive lactic acid production accompanied by a corresponding decline in pH over a 24-hour incubation period. Dornic acidity increased from 14-20°D at inoculation to 32-55°D after 24 hours, while pH decreased from 6.3-6.5 to 4.92-5.35 (Figs. 3, 4 and 5).

Significant strain-dependent variation in acidification capacity was observed. *Enterococcus malodoratus* (S12) demonstrated the highest acidifying efficiency (55°D), whereas *Enterococcus faecium* (S07) exhibited the lowest (30°D). This highlights intraspecific functional divergence, suggesting that strain selection could be strategically leveraged to optimize



T: Time (h) *S: Strain*

S1: Lactococcus lactis subsp. lactis; S13: Lactococcus garvieae; S17: Lactococcus lactis subsp. lactis

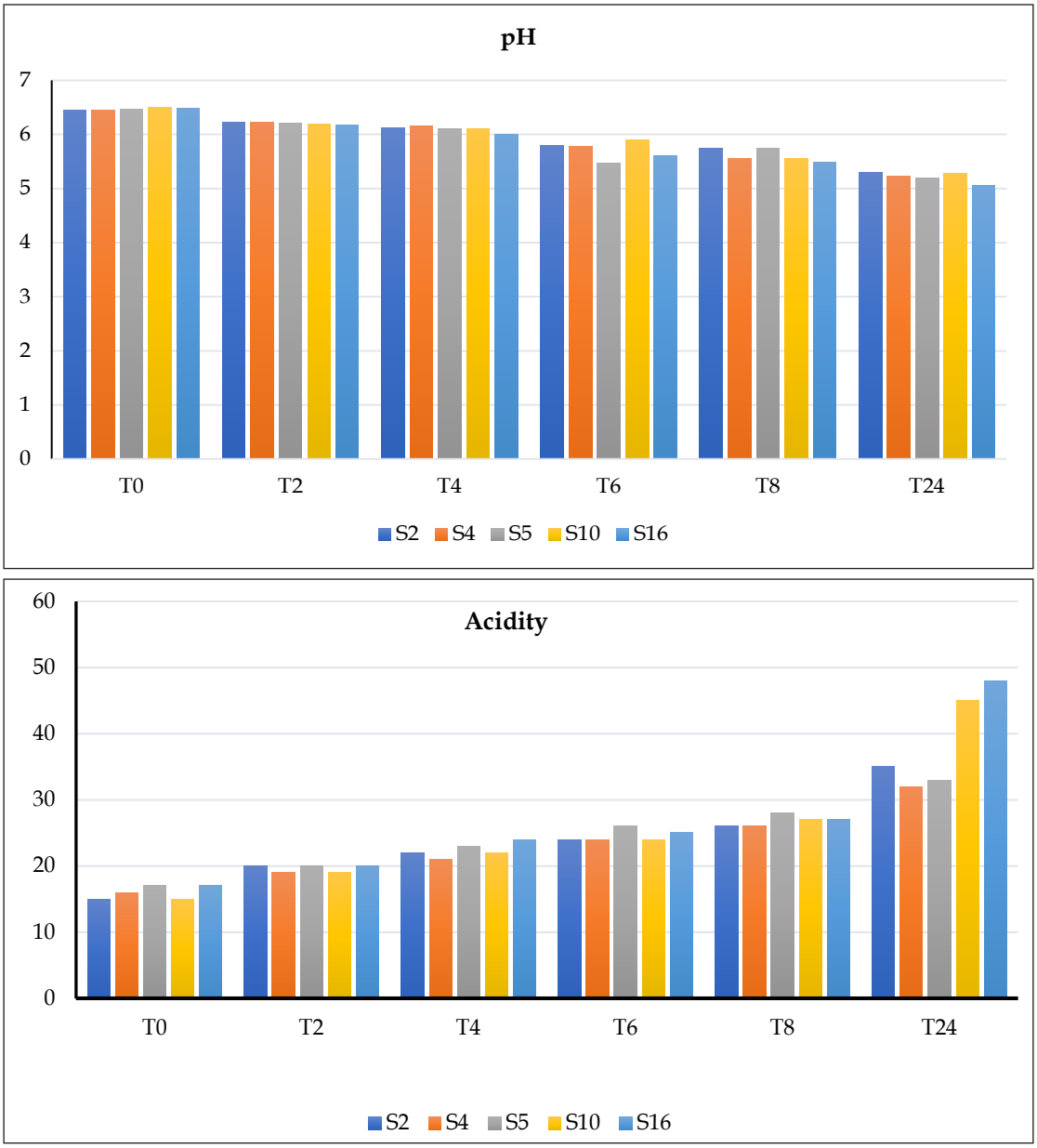
Fig. 3. Results of pH and acidity (°D) evolution for isolated lactic acid bacteria belonging to the genus Lactococcus.

fermentation rate, microbial stability, and product safety in controlled smen production.

Lipolytic activity: None of the isolates exhibited lipolytic activity against Tween 80, confirming that LAB play a minor direct role in lipid hydrolysis. This observation aligns with established evidence that LAB are weakly lipolytic compared with Gram-negative spoilage bacteria. Nonetheless, LAB may contribute indirectly to flavor development by modulating redox balance, producing aroma precursors, and interacting with secondary microflora during maturation.

Processing-driven microbial selection and comparative insights

All analyzed smen samples underwent butter washing, prolonged heat treatment, salting, and sealed storage, creating a selective environment favoring thermotolerant, halotolerant, and stress-resilient LAB. This selective pressure likely underpins the dominance of Enterococcus observed in this study.

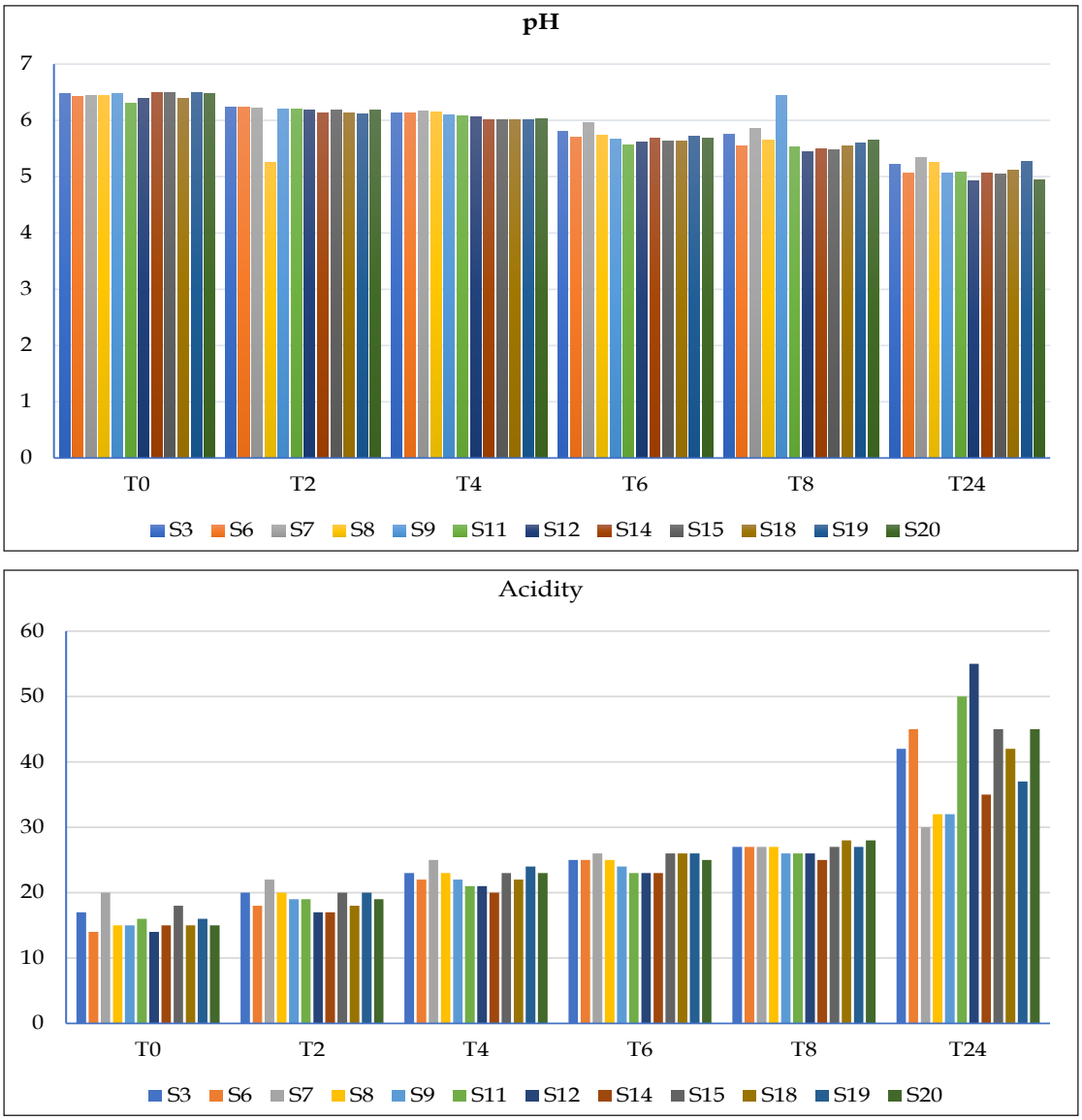


T: Time (h) *S: Strain*
S2: Pediococcus acidilactici; S4: Pediococcus acidilactici; S5: Pediococcus sp.; S10: Pediococcus spp.; S16: Pediococcus pentosaceus
Fig. 4. Results of pH and acidity (°D) evolution for lactic acid bacteria isolated from the genus Pediococcus.

The microbial profile differs notably from Moroccan smen, where mesophilic *Lactobacillus* species frequently dominate due to the absence of cooking, and from camel-milk smen in southern Algeria, where *Lactobacillus* and *Leuconostoc* prevail. These contrasts underscore the strong influence of raw material origin, animal species, processing intensity, and regional climate on LAB community structure and functional potential.

Integrated interpretation and implications

Collectively, the findings demonstrate that traditional Algerian smen supports a LAB consortium dominated by highly stress-tolerant *Enterococcus* species, complemented by *Pediococcus* and *Lactococcus* populations. These bacteria exhibit robust survival capacity, metabolic flexibility, and variable acidification efficiency, traits that likely contribute to microbial stability, product preservation, and sensory maturation.



T: Time (h) *S: Strain*

S3: Enterococcus dispar; S6: Enterococcus faecium; S7:Enterococcus faecium; S8: Enterococcus faecium; S9: Enterococcus faecalis ; S11: Enterococcus faecium; S12: Enterococcus malodoratus; S14: Enterococcus malodoratus; S15:Enterococcus faecium; S18: Enterococcus faecium; S19: Enterococcus spp.; S20: Enterococcus faecalis

Fig. 5. Results of pH and acidity (°D) evolution for lactic acid bacteria isolated from the genus Enterococcus.

Although LAB contribute minimally to lipolysis, their acidification activity and ecological resilience appear to be central to fermentation control and shelf stability. The strain-level functional variability identified in this study suggests opportunities for targeted starter culture development, offering a pathway to enhance quality consistency while preserving the traditional character of smen.

Conclusions

Smen is a dairy product which belongs to the Algerian nutritional heritage and whose consumption is still appreciated to this day by the Algerian consumer. From the microbiological analysis (phenotypic, biochemical and physiological) of the different samples chosen for the isolation and identification of lactic acid bacteria of smen origin, we isolated 20 bacteria belonging to three different genera which are: *Enterococcus* (60%), *Pediococcus* (25%) and *Lactococcus* (15%). All isolated are non-hemolytic and heat-resistant homofermentative immobile shells. They degrade arginine, do not liquefy gelatin and do not use citrate and they lack catalase, cytochrome oxidase and nitrate

reductase. Most of these isolates can be cultured at 45°C, at different pH (4.5, 6.5 and 9.5) and at different NaCl concentrations (2%, 4% and 6.5%). As they can also resist 0.4% potassium tellutite and degrade the following sugars: glucose, fructose, galactose, sucrose, maltose, mannitol, inositol, sorbitol, rhamnose, melibiose, amygladine, arabinose and lactose. According to the results of the study of technological characteristics, not all isolates have lipolytic activity and have an acidifying activity which is variable between the three isolated genera, between species of the same genus and even between the isolates of the same species. The S12 "*Enterococcus malodoratus*" isolate has the best acidifying activity, it secretes a quantity equal to 55°D of lactic acid after 24 hours, while the S07 "*Enterococcus faecium*" isolate has the lowest activity (30°D).

Conflict of Interest

None.

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