



Assessment of the Probiotic and Technological Potential of Three Strains of *Lactobacillus acidophilus* Isolated from Local Dairy Products from Western Algeria

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ABSTRACT

Background: Lactic acid bacteria are widely used in the manufacture of fermented dairy products, due to their specific metabolic activities.

Methods: The main approach of this study is to evaluate the probiotic and technological performance of 03 strains of *Lactobacillus acidophilus* isolated from local dairy products from western Algeria. The evaluation was based, on the one hand, on their in vitro resistance to acidic stress, low pH and bile salts SB, their tolerance to antibiotics and, on the other hand, on their ability to ferment and lactic coagulate cheese milk.

Result: Their ability to withstand the acidic stresses of low pH (2) and SB (1%) clearly demonstrated that the *Lactobacillus acidophilus* species identified is probiotic. The strain's ability to inhibit pathogenic bacteria was confirmed by its interaction with pathogenic strains (*Escherichia coli*, *Pseudomonas*, *Salmonella* and *Staphylococcus aureus*). The 03 strains showed good technological potential, either in culture alone or in mixtures, confirming its crucial dual role in dairy processing and as a probiotic agent with health benefits. Algerian dairy products can be considered a diverse microbial ecosystem and a source of probiotic lactic microflora with nutritional, preventive and therapeutic efficacy.

Key words: Biotechnology, *Lactobacillus acidophilus*, Local dairy products, Probiotic potential.

INTRODUCTION

Lactic acid bacteria have always been present in the human diet and have often been shown to have a beneficial effect on health and in particular on the balance of the intestinal flora (Nagpal *et al.*, 2012; Valdiviezo-Marcelo *et al.*, 2023).

The characterization of lactic acid bacteria from Algerian dairy products has been extensively evaluated in Algerian research centers and laboratories (Dahou *et al.*, 2021). These traditional dairy products host a diverse microbiota, made up of endogenous microbial populations, with functional properties that give them better bioconservation and hygienic, nutritional and organoleptic qualities (Ferdouse *et al.*, 2023; Stefanska *et al.*, 2021).

In recent years, considerable interest has focused on the use of probiotics with technological aptitudes and therapeutic effects (Shokryazdan *et al.*, 2017; Md Mahmudul *et al.*, 2025). Lactobacilli are lactic acid bacteria representative of the beneficial intestinal microbiota for humans and are therefore the most prominent microorganisms used as probiotics (Adriani *et al.*, 2024; Jagadeesh, 2015; Salyers *et al.*, 2004). Probiotics are defined as living microorganisms which, when ingested in adequate quantities, confer beneficial effects on the host (Chaudhary *et al.*, 2019; Wang *et al.*, 2021).

Dairy product manufacturers have also paid increasing attention to exploiting indigenous lactic microflora, selected on the basis of techno-functional criteria, to produce dairy derivatives with probiotic qualities and health benefits.

The aim of this study is to identify high-performance microorganisms that develop probiotic activity in local dairy ecosystems in western Algeria. To achieve this, several

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approaches were developed, including preliminary identification of these microorganisms, evaluation of their selected probiotic effect, in particular by studying their anti-microbial activity, resistance to bile salts and gastric acidity and their biotechnological aptitude for fermentation and lactic coagulation.

MATERIALS AND METHODS

Lactic acid bacteria strain

This study was carried out at the Laboratoire des sciences et techniques de la production animale, Abdelhamid Ibn

Badis University, Mostaganem, Algeria. The experimental study was carried out over 18 months, from May 2023 to October 2024.

Three strains of *Lactobacillus acidophilus* (LBA1, LBA2 and LBA3) were isolated from artisanal dairy products from three different regions of western Algeria. Molecular identification was established using REP-PCR technology. The sequences obtained were compared with those of the reference strain (ATCC 4356) and with those of the GeneBank database using NCBI's Blast Program (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Inoculum preparation

To evaluate the strains through their preserved inoculum, we used the procedure described by Valdiviezo-Marcelo *et al.* (2023). Selected bacterial strains were grown anaerobically for 16-18 h at 37°C in MRS (Man, Rogosa and Sharpe, 1960) broth. Cultures selected for probiotic characterization were those whose biomass reached an OD (optical density assessed by a Genova Bio Jenway UV visible spectrophotometer) of 600 nm, *i.e.* a bacterial count of 10⁸ CFU/ml. These approved cultures were centrifuged for 30 min at 15,000 rpm and 4°C, the pellet was washed with PBS (phosphate-buffered saline) and then resuspended in sterile MRS medium.

In vitro characterization of isolate probiotic properties

Tolerance to acidity and bile salts

The method described by Both *et al.* (2010) was used to determine the acidity tolerance of the species identified. The acid pH of MRS broth was adjusted to 2.0, 3.0, 4.0 and 6.5 using 1M HCl. A 1% volume of young bacterial culture, growing for 14 hours, was inoculated into MRS broth at different pH levels and incubated for 120 min at 37°C, with density evaluation at 600 nm OD.

Tolerance to bile salts was assessed using the method of Ferdouse *et al.* (2023). Young cultures incubated for 14 h (at a dose of 1%) were inoculated into MRS broth supplemented with 0.3%, 0.5%, 1% bile salts and a 0% control. Bacterial density was assessed by spectrophotometry at 600 nm OD over a 24-hour interval at 37°C.

Antimicrobial activity

The antibacterial activity of *Lactobacillus acidophilus* was tested using the well and agar spot diffusion method described by Halder *et al.* (2017) and Tejero-Sarinena *et al.* (2012).

On the one hand, *Lactobacillus* isolates were inoculated punctually in 1st layer on MRS agar plates and incubated for 48 h at 37°C under anaerobic conditions in the presence of CO₂. On the other hand, a 7 ml volume of nutrient agar (0.8% agar) containing 0.5 McFarland of pathogenic indicator cultures used with a count $\geq 10^8$ CFU/ml, respectively of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella enterica* (DSM 17058) were added as a second layer on the plates and

maintained after solidification at 37°C for 24 hours. After this incubation, the diameter (mm) obtained from the inhibitory zone of the 03 strains on each pathogenic indicator culture was measured using an ECO 605D series 150 digital caliper.

Antibiotic susceptibility

The diffusion method was performed according to Wang *et al.*, 2021. Standard disks of erythromycin (10 µg), oxacillin (10 µg), tetracycline (10µg), penicillin (10 µg), ampicillin (10 µg) and streptomycin (10 µg) were used. A volume of 100 µl of a young bacterial culture controlled at an OD of 600nm was spread using a sterile spreader to evenly impregnate the surface of the MRS agar plate, then allowed to dry. 03 discs of each antibiotic were introduced per inoculation plate. Incubation took place under anaerobic conditions for 24 hours at 37°C. After incubation, the zone of inhibition (in mm) of each antibiotic disk was measured.

Technological performance of lactic strains

The technological performance of lactic strains at processing is determined by measuring the total lactic coagulation time according to the method described by IDF (2020) and updated by Dahou *et al.* (2021), this time corresponds to the moment when the first droplets of whey appear (start of whey exudation) on the surface of the lactic gel, also known as coagulum, which becomes rigid and no longer flows along the walls of the experimental tube.

Optimization of lactic coagulation was established using a HÖPPLER® KF 3.2 bead-fall viscometer from German manufacturer Rheotest to measure and trace in real time the firmness of milk gels during lactic coagulation after real-time lactic fermentation (per lactic strain tested with incubation at 37°C). This optimization increases the solids retention rate (dry extract yield%) by determining the optimal gel firmness.

The Dry matter DM retention rate was calculated using the following formula employed by the LSTPA laboratory research team:

$$\text{DM retention rate} = \frac{\text{DM weight of milk} - \text{DM weight of whey}}{\text{DM weight of milk}}$$

Statistical analysis

Data were pre-processed and analyzed using Microsoft Excel 365 ® 16.0. Results are expressed as mean values for the 3 replicates and are compared by analysis of variance for the two criteria studied, probiotic and technological potential (ANOVA 2). Differences are significant when the p decision threshold is <0.05.

RESULTS AND DISCUSSION

In vitro characterization of isolate probiotic properties

Tolerance to acidity and bile salts

The ability of the strains to grow under extreme conditions of initial pH (2; 6.5) and bile salts (0.3; 1%) was tested in MRS broth by measuring their optical density over time

(24h). These strains showed good growth behavior when environmental conditions became more extreme (pH 2 and 1% bile salts). According to Both *et al.* (2010), resistance to pH 2 is a characteristic observed in only a few species of the *Lactobacillus acidophilus* genus. Indeed, tolerance to gastrointestinal conditions, in this case to acidity and the presence of bile salts, has highlighted an essential property in the characterization of this probiotic strain. The results show in Fig 1 and 2 that the lower the pH, the weaker the growth OD of the *L. acidophilus* strains tested (LBA1, LBA2 and LBA3). The OD fell from an average of 0.47 at pH 6.5 to 0.14 at pH 2. Moreover, at 0% SB bile salts, growth was optimal, with an OD of 0.310, compared with 0.063 at an extreme dose of 1%.

Despite the visual differences in growth density of the 03 strains shown in Fig 1 and 2, resistance to the more hostile conditions of low pH (2) and high SB content (1%) was observed.

These results corroborate those of Begely *et al.* (2006), who showed that this strain isolated from a dairy product possessed enzymes capable of hydrolyzing over 35% of bilirubin salts. Their growth may have been inhibited by the overly acidic medium ($\text{pH} \leq 2.5$). Acid treatment strongly influences the growth of certain probiotics, as shown by the work of some authors (Suskovic *et al.*, 2000; Wang *et al.*, 2021) who observed an absence of growth of certain lactic and probiotic bacterial strains on MRS broth after treatment with acid and beef bile. Qualitative results also confirmed that after culture in MRS broth (pH 6.5 and 1% SB; pH 2 and 1% SB) and plating on agar, *Lactobacillus acidophilus* showed good viability with colonies on MRS agar after 24 hours incubation. This clearly shows that the 03 strains are resistant to adverse and extreme conditions. These results are identical with those obtained by Adriani *et al.* (2024), Begely *et al.* (2006) and Naggal *et al.* (2012) who observed stability of lactobacilli, notably *L. acidophilus*,

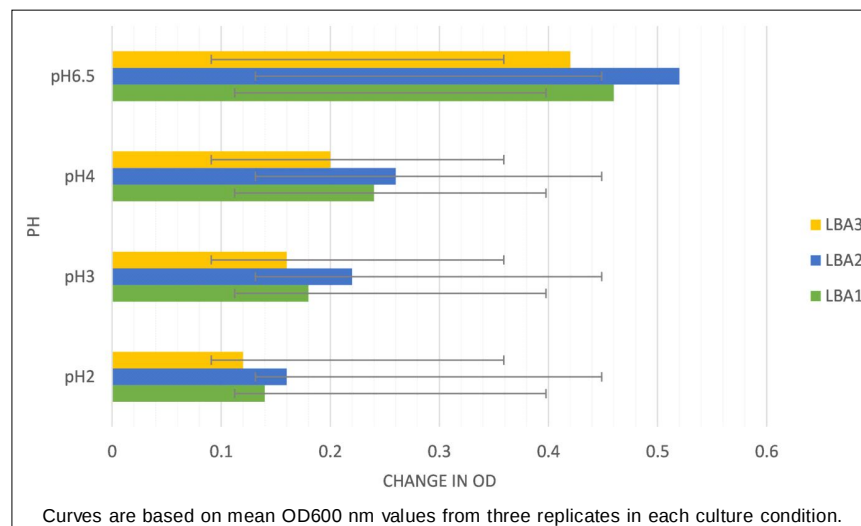


Fig 1: Evolution of optical density at 600 nm over 24 hours for the three strains in MRS broth; Anaerobic 37°C; at different acid pH.

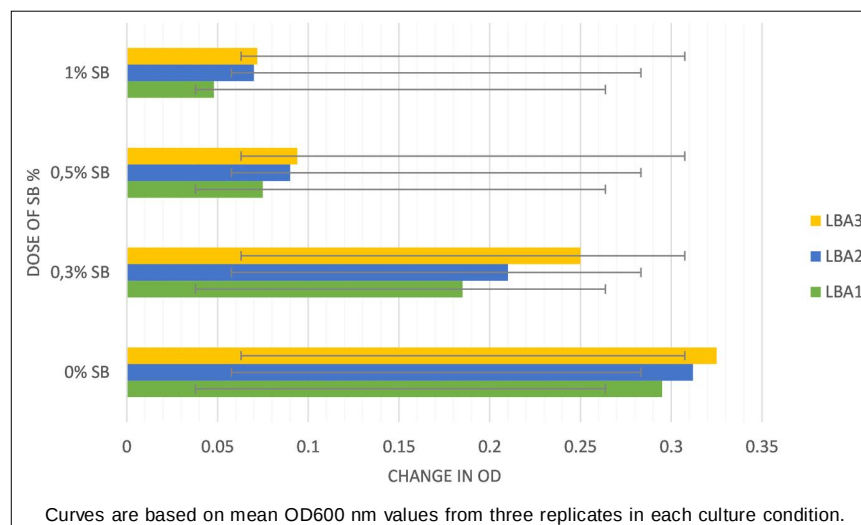


Fig 2: Evolution of optical density at 600 nm over 24 h of the three strains in MRS broth; Anaerobic 37°C; at different doses of SB bile salts.

in a medium similar to intestinal conditions at pH 3 in the presence of 1% SB.

Antimicrobial activity

The three strains studied were able to inhibit *E. coli*, *Pseudomonas*, *Salmonella* and *Staphylococcus*. The diameters of the inhibition zones are expressed in mm in Table 1.

Antimicrobial activity (x) was calculated on the basis of the formula used by the LSTPA research laboratory where $x = D - d$, D represents the diameter of the inhibition zone and d the diameter of the well. Antimicrobial activity (x) was characterized and classified according to inhibition zone diameter and described as low ($x < 4$ mm), medium ($x = 4$ –25 mm) and high ($x > 25$ mm).

The 03 *Lactobacillus acidophilus* strains showed significant inhibition zones, underlining their high inhibitory power. Bacteria classified as (LBA1) (LBA2) and (LBA3) showed a zone of inhibition classified as medium on all four pathogens.

Pathogen inhibition is a key property sought in probiotics (Kumar *et al.*, 2016). The strains tested showed a zone of inhibition with mean diameters ranging from 9 to 21 mm around the pathogens *E. coli*, *Pseudomonas*, *Salmonella* and *Staphylococcus*. These results are superior to those of Tejero-Sarinena *et al.* (2012), who showed that a strain of *L. acidophilus* inhibited the same indicator microorganisms with small diameters. These differences could be due to the specificity of the ecosystem in which these strains were isolated (Geetha *et al.*, 2015; Samot and Badet, 2013). The difference in results obtained can also be explained by the method used to perform the test. Indeed, the inhibitory activity highlighted by these authors is based on the use of culture supernatants, whereas in our case, it involves the use of inhibition by diffusion of a new culture of *Lactobacillus acidophilus* in its specific MRS medium to a nutrient medium suitable for the pathogenic cultures used. This is to avoid the antagonism common to

lactic acid bacteria derived from fermented products. The inhibition diameter values found in our study are practically of the same order as those found by recent studies on the inhibitory power of lactic acid bacteria strains isolated from local dairy products (Md Mahmudul *et al.*, 2025).

Antibiotic susceptibility

As part of the ongoing characterization of our potentially probiotic strains, antibiotic susceptibility tests were carried out. For each antibiotic tested, the diameters of the zones of inhibition measured were used to define resistant and sensitive strains.

Antibiotic sensitivity is one of the most important safety criteria in the selection of probiotic strains (Baquero *et al.*, 2013; Md Mahmudul *et al.*, 2025). In our study, our 03 strains were tested against six (6) different antibiotics: ampicillin, erythromycin, oxacillin, penicillin, streptomycin and tetracycline. The diameters (mm) of inhibition are shown in Table 2. The zone of inhibition is illustrated by the clear zones (opaque halos) around the antibiotics tested and is shown in the Fig 3. It is clear that *Lactobacillus acidophilus* is sensitive to the antibiotics used.

According to the CDC, 2013 (Centers for Disease Control and Prevention), a bacterium is resistant to an antibiotic if the diameter of the zone of inhibition is less than 8 mm, intermediate if the diameter is between 8 and 22 mm. Above 22 mm, the bacterium is considered sensitive to the antibiotic. The inhibition zone diameters obtained in Table 2 exceed 22 mm. Based on this criterion, the 03 *Lactobacillus acidophilus* strains are fully sensitive to the antibiotics tested.

These results are in line with those of Smith and Coast, (2013), who showed that with a diameter > 5 mm, dietary supplements, mainly lactic acid bacterial strains, were sensitive to erythromycin and streptomycin. The only difference from their study was in the penicillin groups, which could be explained by the antibiotic concentrations used. In fact, these researchers used 1 µg of antibiotic, a concentration ten times lower than ours (10 µg).

Table 1: Average assessment of antimicrobial activity by determining zones of inhibition in mm.

PM \ LBA	E. coli (mm)	Pseudomonas (mm)	Salmonella (mm)	Staphylococcus (mm)
LBA1 (*)	20.82±0.03	15.14±0.01	10.32±0.03	20.32±0.03
LBA2 (*)	19.78±0.02	12.68±0.02	13.08±0.02	16.02±0.03
LBA3 (*)	18.23±0.02	17.02±0.03	09.98±0.02	15.12±0.03

PM: Pathogen microorganism, LBA: *Lactobacillus acidophilus*,* significant ($p < 0.05$), n=3.

Table 2: Assessment of *Lactobacillus acidophilus* sensitivity to antibiotics by determining zones of inhibition.

LBA \ Antibiotic	Ampicillin	Erythromycin	Oxacillin	Penicillin	Streptomycin	Tetracycline
LBA1 (NS)	38.02±0.3	32.57±0.3	23.77±0.3	31.16±0.4	29.77±0.3	24.98±0.2
LBA2 (NS)	37.54±0.6	32.38±0.2	23.98±0.2	31.17±0.3	29.51±0.4	24.78±0.2
LBA3 (NS)	37.77±0.3	32.62±0.3	24.17±0.3	30.98±0.2	29.82±0.3	24.92±0.3

LBA: *Lactobacillus acidophilus*, NS: Not significant ($p > 0.05$), n=3.

Technological performance of lactic strains

Standardized total lactic coagulation STLCT took place over 18 hours for the 03 strains tested. According to the results obtained in Table 3.

- Strain LBA1 had an average total lactic coagulation time of 18 hours and 28 minutes with a lactic curd viscosity of 978 cp, i.e. a corresponding dry extract (DE) of 11.94% and a loss of 0.56% (< at 1%).
- Strain LBA2 had an average total lactic coagulation time of 18 hours and 15 minutes with a lactic curd viscosity of 997 cp and an DE of 12.23%, representing a loss of 0.27% (< at 1%).
- Strain LBA3 had an average total lactic coagulation time of 18 hours and 19 minutes with a lactic curd viscosity of 986 cp and an DE of 12.1%, i.e. a loss of 0.4% (< at 1%).

The blend of 03 strains gave an average total lactic coagulation time of 18 hours and 13 minutes with a lactic curd viscosity of 1002 cp and an DE of 12.3%, i.e. a loss of 0.2% (< at 1%).

According to the recommendations of the IDF (2020) and the results on similar studies obtained by Dahou *et al.* (2021), the bacterial strains are intended for secondary fermentation: i.e. for proteolytic activity for cheese maturation during ripening.

These bacterial strains can also be used for primary fermentation, i.e. for the manufacture of yogurt-type milks in association with a *Streptococcus thermophilus* strain:

- As follows: 60% *Streptococcus thermophilus* and 40% *Lactobacillus acidophilus* to obtain a yogurt-type fermented milk with firm lactic curd.

- Or 40% *Streptococcus thermophilus* and 60% *Lactobacillus acidophilus* for a fermented milk with stirred lactic curd.

Normalized firmness for lactic coagulation is calculated for a viscosity of between 850 and 1100 centipoises (cp) for milk standardized in fat-free dry extract FFDE at 12.5% and is used as the target firmness for fermented milks. Stable lactic curd firmness favors good solids retention, averaging 11.5 to 12% for an initial milk DM content of 12.5%, i.e. a loss rate of no more than 1%.

Experimental tubes in which firm milk gels were obtained after more than 18 hours of fermentation, i.e. between 18 H-13min and 18 H-28 min, showed higher solids retention rates and a low loss rate of between 0.2 and 0.56%.

Solids retention rate is a better indicator of coagulation performance and improved overall yield of milk used (Xiuju and Zhengtao, 2022). According to Dahou *et al.* (2021), for the optimization of total lactic coagulation of milk at 12.5% DM. The viscosity of the resulting lactic curd should be between 850 and 1100 cp. Viscosity will guide processing technology, i.e. cutting the curd to a controlled firmness for better dry matter DM retention and milk yield.

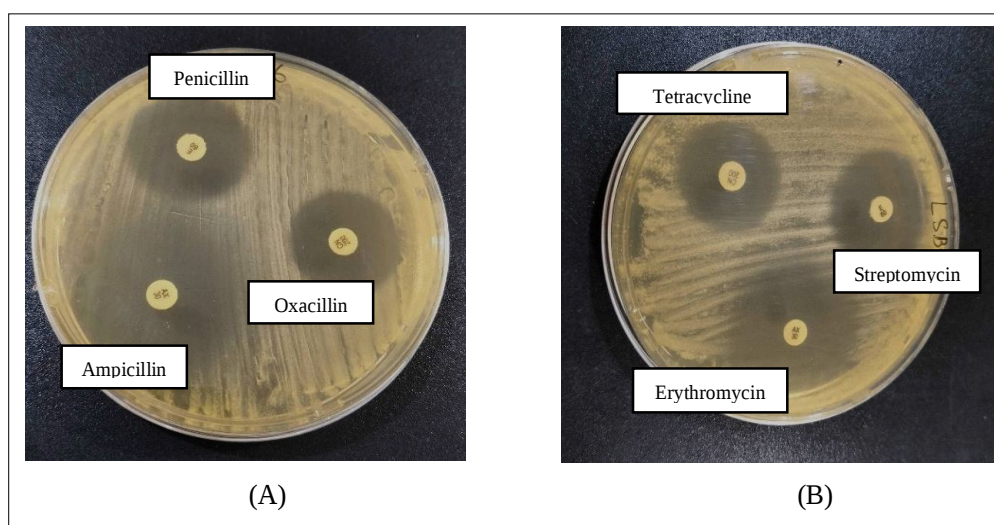


Fig 3: Zones of inhibition for the six antibiotics tested. (A): Ampicillin, Oxacillin and Penicillin ; (B): Erythromycin, Streptomycin and Tetracycline.

Table 3: Results of viscosities (in cp) obtained by strain tested at total lactic gel firmness (at total lactic coagulation).

Strain	12H	13H	14H	15H	16H	17H	18H	Over 18H
LBA1 (*)	295	328	410	570	625	775	832	978
LBA2 (*)	310	335	430	580	630	782	844	997
LBA3 (*)	302	332	423	575	628	775	838	986
B (*)	318	337	438	585	635	790	848	1002

LBA: *Lactobacillus acidophilus*, B (The Blend of the three strains),* significant ($p < 0.05$).

Statistical analysis

The statistical study carried out on the 02 evaluation criteria (probiotic and technological potential) of *Lactobacillus acidophilus* lactic strains, previously standardized to cell growth concentrations, gave significant results ($p > 0.05$) for their survival in vitro over an incubation period and in hostile acidity and bile salt environments and non-significant ($p > 0.05$) for their viability in the presence of antibiotics. Analysis of the results showed progressive adaptation to increasing bile thresholds (up to 1% SB), with tolerance to acid pH (ranging from 4 to 2). Generally speaking, the levels of resistance observed are sufficient for these bacteria to survive gastrointestinal transit *in vivo* and to settle easily in the digestive tract.

Furthermore, the antibacterial properties of the strains were also expressive with cultures that ensured the diffusion of their metabolites, *i.e.* substances with a pronounced antibacterial effect against the 04 pathogenic microorganisms tested. The zones of inhibition were highly significant for dangerous and rapidly spreading pathogens.

The interest of these acidifying bacteria for bio-industries lies essentially in their biotechnological properties in fermentation and lactic coagulation (Dahou *et al.*, 2021; Xiuju and Zhengtao, 2022). The lactic acid production profiles obtained for lowering to isoelectric pH and obtaining a compact biomass were highly significant ($p < 0.05$). The lactic coagulation configuration obtained, within the tuned lactic fermentation times, met the standards for viscosity and solids retention in the lactic curd mass for the 03 *L. acidophilus* strains.

CONCLUSION

At the end of this research, we have a body of knowledge on the probiotic performance of 03 *Lactobacillus acidophilus* lactic strains isolated from local dairy products from western Algeria. The various results and discussions that have emerged throughout this study open up prospects for the development of promising *in vivo* clinical evaluation in human health. In particular, for the fight against gastrointestinal and metabolic diseases and on the other hand for dairy technology, thanks to controlled biofermentation and lactic coagulation, to produce dairy derivatives with typical nutritional, sensory and hygienic qualities.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the

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Conflict of interest

The authors declare no conflict of interest.

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