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ORIGINAL PAPER

Investigating the biotechnological potential of lactic acid bacteria strains isolated from different Algerian dairy and farm sources

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Abstract

Currently, consumption of spontaneously fermented milks is common in Algeria, making it a feasible source of diverse lactic acid bacteria (LAB) with the potential to be used as adjunct cultures to improve quality and safety of fermented dairy products. In this context, to select eligible indigenous strains which could be applied as bioprotective and/or starter cultures, the present study aimed to characterize the genomic variability, biotechnological potential, and safety of thirty-eight LAB isolated from Algerian dairy and farm sources of western Algeria. The isolates were unequivocally identified by 16S rRNA gene and fingerprint-based methods. The following species were identified: *Enterococcus faecium* ($n=15$), *Enterococcus durans* ($n=2$), *Enterococcus hirae* ($n=2$), *Enterococcus lactis* ($n=1$), *Lactiplantibacillus plantarum* ($n=6$), *Lactococcus lactis* ($n=4$), *Levilactobacillus brevis* ($n=3$), *Lacticaseibacillus paracasei* ($n=3$), *Lacticaseibacillus rhamnosus* ($n=1$), and *Pediococcus acidilactici* ($n=1$). Among the strains, three of them, *L. lactis* LGMY8, *Lb. plantarum* LGMY30 and *Lb. paracasei* LGMY31 were safe and showed some valuable biotechnological properties, such as high acidification, proteolytic activity, EPS production, and inhibition of undesirable bacteria that made them powerful candidates to be used as starter.

Keywords Dairy milk · Lactic acid bacteria · Safety assessment · Technological properties · Bioprotective cultures

Introduction

Dairy products are the second most consumed staple food in Algeria, following cereals (Hales and Torry 2018). Due to the local insufficient production of milk caused by the absence of a robust national milk industrial chain (Sraïri et al. 2013), Algeria importations arrive to 250,000 up to 280,000 tons of powdered milk per year (Kardjadj and Dachung Luka 2016). Other limitation to technological advancement of milk self-sustaining is the high level of microbial contamination originated from poor hygienic practices in all stages of the chain, starting by the milking, collection, transporting/distribution, and storage of milk.

Previous studies on Algerian milks showed that they are reservoirs of several LAB, such as *Lacticaseibacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Pediococcus* and *Lactococcus* spp. with antifungal and antibacterial activity (Mechai et al. 2020), which may be exploited to ensure the safety of fermented foods.

The isolation and characterisation of indigenous microbial diversity is a key step to design tailored starter cultures for artisanal/traditional fermented food that increase the safety and quality of such highly appreciated foodstuffs (Capozzi et al. 2020; Saidi et al. 2020). In Algeria arid regions, camel's milk for example is considered as one of the most important sources of dairy products for human diet with potential therapeutic effects.

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Specific sensory characteristics are generated from LAB metabolic pathways, resulting in a diversity of aromatic compounds. They convert the sugar into lactic acid, resulting in the rapid acidification of raw material, and produce other metabolites, such as ethanol, diacetyl, acetate, and acetaldehyde that improve the flavor, texture, taste, storage, and safety of the end products (Leroy and Vuyst 2004; Perin et al. 2017) and might be selected to improve or replace currently used starters and adjunct cultures (Brandsma et al. 2008; Alegria et al. 2016).

The LAB also contribute to the proteolysis of cheese, as they can degrade the products derived from the rennet action on the casein (peptides of high and low molecular mass). (Herreros et al. 2003) They are also the object of intensive international researches for their ability to produce several antimicrobial compounds, such as bacteriocin (De Vuyst and Leroy 2007; Reis et al. 2012; Benmechernene et al. 2013), for their essential role in the food fermentation and degradation of protein that lead to the synthesis of a wide range of compounds, such as organic acids, peptides, aromatic compounds and exopolysaccharides (Saidi et al. 2019; Mende et al. 2016).

Obviously, the selection of potential starter cultures must focus not only on their functional properties but also on the absence of production of undesirable factors, such as biogenic amines (BA) and antibiotic resistance (AR) genes. Antibiotic resistant bacteria constitute a serious problem for the health of both humans and animals (Berendonk et al. 2015), and fermented foods could spread AR genes along the food chain to the human gastrointestinal tract (Founou et al. 2016). BAs are low molecular weight nitrogenated compounds which can accumulate in foods though the microbial decarboxylation of certain amino acids (Linares et al. 2011). Certain LAB strains are the main responsible for production and accumulation of BA in dairy products (Linares et al. 2012), thus assessment of the ability to produce BA by potential protective LAB starters is essential to improve food safety and the consumers health (Ladero et al. 2017).

The bioprospecting of recovering new safe protective LAB strains from Algerian milks configures an excellent strategy to save the local biological heritage and to maintain the geographical identity of the obtained fermented product (Saidi et al. 2020; Merabti et al. 2019).

For these reasons, the aim of the present study was to unambiguously identify thirty-eight indigenous bacteria collected from dairy Algerian milks, assess their genetic diversity through molecular fingerprinting, evaluate their technological potential and safety.

Materials and methods

Bacterial strains, sample collection and growth conditions

A total of thirty-eight LAB, previously isolated from different milk sources (camel, cow, sheep and goat milk), from environmental samples (pollen, olive oil, traditional cheese) and from laboratory collection collected from nine areas of south and northwestern of Algeria (Tindouf, Bechar, Adrar, Tiaret, Oran, Tlemcen, Saida, Jijel, Kabilie) (Table 1). Coccus-shaped isolates were cultured in M17 medium (Oxoid, UK) supplemented with 0.5% glucose and incubated at 32 ºC for 24–48 h. Rod-shaped strains were grown in MRS medium (Oxoid) incubated in anaerobic conditions under a 10% H_2, 10% CO_2 and 80% N_2 atmosphere in a MACS MG-500 anaerobic chamber (Don Whitley Scientific, West Yorkshire, UK) at 37 °C for 48 h. The strains were stored as frozen stocks at − 80 °C in the respective culture medium supplemented with 20% (v/v) glycerol.

Molecular identification and typing

DNA extraction

Genomic DNA was extracted from 1.5 mL overnight cultures. In brief, cells were harvested by centrifugation (12,000 rpm for 2 min), washed with distilled water, and then resuspended in a lysozyme solution (30 µg/mL) supplemented with mutanolysin (100 U/mL). After incubation at 37° C for 30 min, proteinase K (20 µg/mL) was added and a second step of incubation at 55 °C for 30 min was carried out. Genomic DNA was purified using the GenElute Bacterial Genomic DNA kit (Sigma-Aldrich, USA), following the manufacturer's recommendations. DNA yield and purity were quantified using the Nanodrop ONE UV–Vis Spectrophotometer (Thermo Scientific, USA). DNA was stored at − 20° C for downstream analysis.

Molecular identification of the isolates

Total DNA was used as template to amplify the universal region of the 16S rRNA gene by PCR using the primers 27F and 1492R, according to Lane (1991). PCR amplicons were examined by 1.2% (w/v) agarose gel stained with ethidium bromide (0.5 µg/mL) and visualized by GelPrinter plus (TDI, Spain). PCR products were purified by theATP™ Gel PCR Fragment DNA Kit (ATP Biotech Inc., Taipei, Taiwan) and delivered to Macrogen (Amsterdam,The Netherlands) for Sanger sequencing. Lactobacilli were identified by species-specific PCRs according to the protocols described

Table 1 List of LAB isolates with their isolation source and 16S rRNA gene-based identification

Source	Isolate	16S rRNA similarity % (nt/nt)[a]	Identification at species level	Accession number[b]
Camel milk	LGMY3	100 (1441/1441)	*P. acidilactici*	MZ346597
	LGMY9	100 (1321/1321)	*Lpb. plantarum*	MZ333272
	LGMY23	100 (1375/1375)	*Lpb. plantarum*	MZ333455
	LGMY29	99 (1380/1386)	*Lpb. plantarum*	MZ333463
	LGMY31	99 (1403/1410)	*Lcb. paracasei*	MZ333474
Cow milk	LGMY4	99 (1316/1318)	*L. lactis*	MZ333219
	LGMY8	99 (1347/1349)	*L. lactis*	MZ333271
	LGMY34	100 (1383/1383)	*Lcb. rhamnosus*	MZ346604
Sheep milk	LGMY17	100 (1334/1334)	*L. lactis*	MZ333282
	LGMY32	99 (1412/1419)	*Lcb. paracasei*	MZ333532
	LGMY36	99 (1351/1358)	*L. lactis*	MZ334469
Goat milk	LGMY16	99 1361/1362)	*Lpb. plantarum*	MZ333431
Cheese	LGMY30	99 (1398/1406)	*Lpb. plantarum*	MZ333468
Lab collection	LGMY26	99 (1374/1381)	*Lvl. brevis*	MZ333453
	LGMY27	99 (1388/1390)	*Lpb. plantarum*	MZ333459
	LGMY35	99 (1390/1397)	*Lcb. paracasei*	MZ334403
Plant material (olive oil-pollen)	LGMY21	99 (1401/1407)	*Lvl. brevis*	MZ333446
	LGMY33	99 (1380/1383)	*Lvl. brevis*	MZ333535

[a]nt/nt: nucleotides/nucleotides

[b]16S rRNA gene accession number at the National Center for Biotechnology Information (NCBI)

for *Lb. plantarum* group (Torriani et al. 2001), *Lb. casei* group (Bottari et al. 2017) and for the *Lb. brevis* species (Guarneri et al. 2001).

Sequence identities were analyzed using BLAST. The 16S rRNA gene sequences were deposited in the NCBI database. The unrooted phylogenetic tree was constructed to determine the closest LAB species by the neighbor-joining method (Felsenstein 1985).

Typing of isolates

Genetic fingerprinting of the isolates was assessed by rep-PCR using the primer $(GTG)_5$ (Iacumin et al. 2006) following the previously reported protocol of Versalovic et al. (1994). PCR amplicons were electrophoresed in 1.5% (w/v) agarose gels for 150 min, and then revealed on UVITEC (UK). The O'GenRuler DNA ladder Mix (Thermo Scientific) was used as molecular size marker Digitalized images were analyzed using the Uvitec Fire Reader Acquisition System and the dendrogram was constructed by the UVIB and Map software (Uvitec, UK).

Technological and functional characterisation

Acidifying activity

Acidifying activity was determined in reconstituted, sterile skim milk (Sigma-Aldrich), according to the protocol of Olasupo et al. (2006). Briefly, single colonies of each strain grown on MRS agar plates incubated at 37 °C for 48 h were pickled and inoculated in skim milk. Then, 1 mL of overnight milk cultures was inoculated in 10 mL of skim milk. Incubation was performed at 37 °C for up to 48 h, and pH variations were measured at 24 and 48 h using a pH-meter (Crison Instruments S.A., Spain). Visual inspection of the clotting regarding whey drainage, curd firmness, presence of gas bubbles and curd breaking was also recorded. The assay was performed in triplicate.

Production of volatile compounds in milk

Volatile compound analysis was performed after growth of the LAB in screw-cap tubes at 37 °C for 48 h in UHT milk supplemented with cyclohexanone (3.6 µg/mL) as internal standard. Separation and quantification of the volatiles compound were performed by headspace-gas chromatography-mass spectrometry (HS-GC–MS), using Agilent apparatus (Agilent Technologies, USA) equipped with a capillary column DB-WAXetr (60 m × 0.25 mm × 0.25 µm). Sample preparation and gas chromatographic separation were performed as described by Salazar et al. (2008). Compounds were quantified as the normalized value of their chromatogram peak areas; the internal standard was given a value of 100. The experiment was performed in duplicate.

Antibacterial activity

The capacity of LAB isolates to produce antimicrobial substances was determined by the agar well-diffusion assay as described in Saidi et al. (2020). *Lb. parabuchneri* St2A, *Listeria innocua* CECT 906 ^{T}, *Micrococcus luteus* NCBI 8166, and *Lactococcus lactis* subsp. *cremoris* MG1363 were used as target strains. Supernatants from overnight cultures, in duplicate, of the tested strains were adjusted to pH 7.0 with 0.1 M NaOH and filtered through a 0.20 µm pore diameter membrane (Millipore). Twenty µL aliquots of each supernatant were placed in wells excavated in the agar plates and were incubated at 37 °C for 24–48 h. The clear inhibition zone around the well was measured in mm. A halo above 8 mm was considered a positive result (Saidi et al. 2020).

Proteolytic activity

Proteolytic activity of whole cells in milk was determined using the O-phthaldialdehyde (OPA) test as previously described (Church et al. 1985). In summary, the increase in optical density at 340 nm (OD_{340}), relative to the control, was determined using the Cary 60 UV–Vis Spectrometer (Agilent Technologies, Santa Clara, CA, USA). The OPA solution contained: 2.5 mL of 20% (w/v) SDS, 25 mL of 100 mM sodium tetraborate (RIEDEL Germany), 40 mg of OPA (Fluka, Biochemika) (previously dissolved in 1.0 mL methanol), 100 µL of 2-mercaptoethanol (BIO RAD), and distilled water up to a 50 mL final volume. The samples were incubated with 0.75 M trichloroacetic acid (TCA) (Fisher Bio Reagents) in a proportion of TCA/sample of 1:3 at 4 °C for 30 min and centrifuged at 5000 rpm for 10 min. A 50 µL supernatant aliquot of this mixture was added to 1.0 mL of OPA reagent and incubated at room temperature for 20 min, then read at the spectrophotometer. Proteolytic activity was arbitrarily expressed as µg of leucine (Leu) released/mL using a standard curve of L-leucine (Sigma Chemical Co).

Exopolysaccharide production

Overnight cultures of isolates were spotted (5.0 µL) on the surface of MRS plates supplemented with 0.08 g/L of ruthenium red (Sigma-Aldrich). After incubation at 37 °C for 48 h, exopolysaccharide (EPS) producing strains gave white colonies, while non-producers appeared as red colonies (Kersani et al. 2017).

Safety assessment

Antibiotic resistance assay

Antibiotic susceptibility was assessed by the disk diffusion method, as described by Anisimova et al. (2017). In brief, all overnight cultures of strains were diluted in 0.85% saline solution to obtain a standardized turbidity equivalent to McFarland scale 0.5. Aliquots of these suspensions were pour-plated in Muller-Hinton agar medium plates. Antibiotic discs for tetracycline (30 µg), vancomycin (30 µg), gentamycin (10 µg) and erythromycin (15 µg) (Bio-Rad, Marnes-la- Coquette, France) were dispensed onto the inoculated plates. After 48 h incubation at 37 °C in anaerobic conditions, inhibition halos were measured in mm (means ± SD of 3 trials) and interpreted as susceptible (S), moderately susceptible (MS), or resistant (R), according to Melo et al. (2017).

Detection of BA-producing genes

The presence of the amino acid decarboxylase genes involved in the production of tyramine [*tdcA,* encoding the tyrosine decarboxylase from the tyrosine decarboxylase cluster (TDC)], histamine [*hdcA,* encoding the histidine decarboxylase from the histidine decarboxylase cluster (HDC)] and putrescine [*aguD-aguA* genes from the agmatine deiminase (AGDI) cluster] was checked by PCR. For that we used the primer pairs *tdc1* and *tdc2* (Fernández et al. 2004), *hdcDG-F* and *hdcDG-R* (Diaz et al. 2016), and *AgmSq1* and *AgmSq2* (Linares et al. 2011), respectively. Positive controls were performed using total genomic DNA obtained from different BA-producing strains: *Enterococcus faecalis* V583 for tyramine and putrescine (via AGDI) (Ladero et al. 2015), and *Lentilactobacillus parabuchneri* IPLA11150 for histamine (Diaz et al. 2016). PCR products were visualized after gel electrophoresis as stated above.

Biogenic amine production

The ability to produce the BA tyramine, histamine and putrescine was evaluated in the isolates showing PCR positive results for the presence of BA-producing genes following the protocol described by Ladero et al. (2015). Briefly, lactococci were grown at 32 °C in GM17 (Oxoid) while lactobacilli were grown in anaerobiosis at 37 °C for 24 h in MRS broth, both supplemented with either 1 mM histidine, 1 mM tyramine or 1 mM agmatine. BA production in culture supernatants was analyzed by Ultrahigh Performance Liquid Chromatography (UHPLC) in a Waters H-Class Acquity UPLC apparatus with a UV detector (Waters, USA) controlled by Empower 2.0 software (Waters), following the protocol described by Redruello et al. (2013).

Results

Molecular identification and typing of LAB isolates

Thirty-Eight LAB isolates from different Algerian dairy and farm sources were identified through 16S rRNA gene sequencing. Based on BLAST analysis, the isolates were preliminarily identified as *Enterococcus faecium* ($n=15$), *Enterococcus durans* ($n=2$), *Enterococcus hirae* ($n=2$), *Enterococcus lactis* ($n=1$), *Lactiplantibacillus plantarum* group ($n=6$), *Lactococcus lactis* ($n=4$), *Lacticaseibacillus casei* group ($n=4$), *Levilactobacillus brevis* ($n=3$), and *Pediococcus acidilactici* ($n=1$) with a similarity value over 99% (Table 1). Since safety aspects related to enterococci have raised questions regarding their use in foods or as probiotics (Berendonk et al. 2015), they were excluded from further analysis.

To identify closely related lactobacilli isolates at species level, species-specific PCRs were performed. Results, showed in the Online Resource 1, allowed the unequivocal identification of *Lpb. plantarum* (isolates LGMY9, LGMY16, LGMY23, LGMY27, LGMY29, LGMY30), *Lvl. brevis* (LGMY21, LGMY26 and LGMY33) and within the *Lcb. paracasei* group, PCR differentiated the isolates of *Lcb. paracasei* (LGMY31, LGMY32 and LGMY35) from *Lcb. rhamnosus* LGMY34 (Table 1).

To highlight genotypic differences among isolates, Rep-PCR and cluster analysis were performed. The dendrogram depicted in Fig. 1 differentiated six groups that coincided with the identification at species level. Further, different band patterns were found at the intraspecific level, revealing the presence of sixteen unique profiles associated to single strains (Fig. 1). However, two isolates of *Lpb. plantarum* (LMGY16 and LMGY29) and two of *L. lactis* (LMGY4 and LMGY36) showed the same profile, even though they were isolated from different sources.

Query coverage of the LAB identification results obtained using BLAST was 99–100%. The partial sequences of isolates were deposited in the GenBank and the accession numbers were reported in Table 1. A phylogenetic tree was constructed using the neighbor-joining method (Fig. 2).

Technological and functional characterization

Acidifying activity

The UHT milk acidification assay (Table 2) showed that all strains were able to grow in milk, and provoked milk clotting after 24 h at 30 °C. After 48 h fermentation, pH values between 2.65 and 2.70 were reached in the vats inoculated with *Lcb. paracasei* LGMY31 and *Lpb. plantarum* LGMY30, *Lvl. brevis* LGMY21 and LGMY33, and *Lcb. paracasei* LGMY31, LGMY32 and LGMY35, resulting in the production of a stable clot. Furthermore, in the vats inoculated with *Lvl. brevis* LGMY33, the gas production was considerable and jeopardized the clot structure resulting in a strong whey drainage. The strains *L. lactis* LGMY17 and LGMY36 acidified the UHT milk more rapidly than the other two lactococcal strains, and reached lower pH values after 48 h.

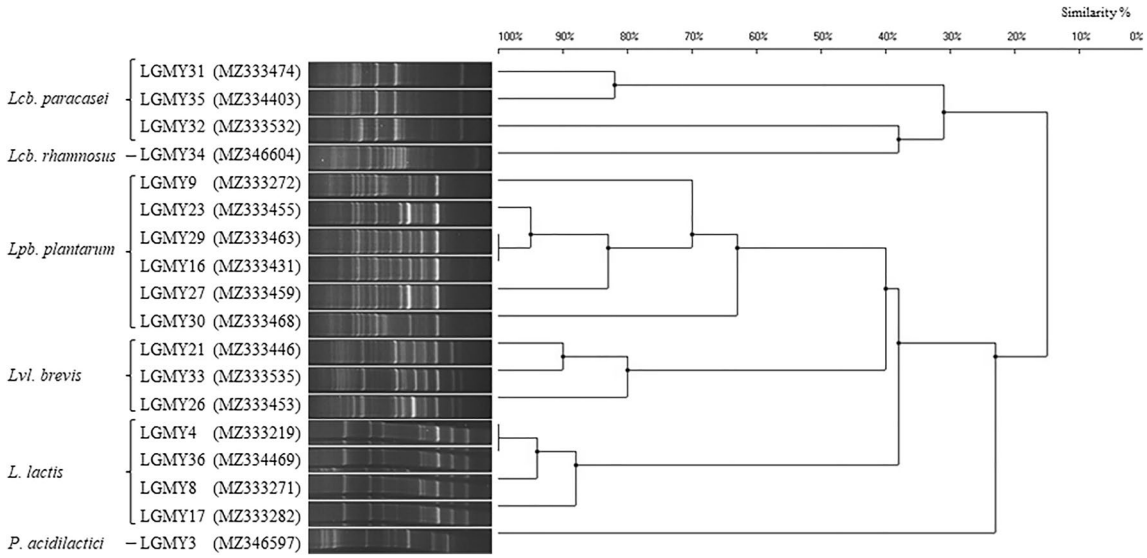

Fig. 1 Dendrogram showing the genetic similarities between LAB isolates based on $(GTG)_5$-PCR fingerprinting. The cluster analysis of genetic distances was performed with the unweighted pair-group method using arithmetic averages (UPGMA)

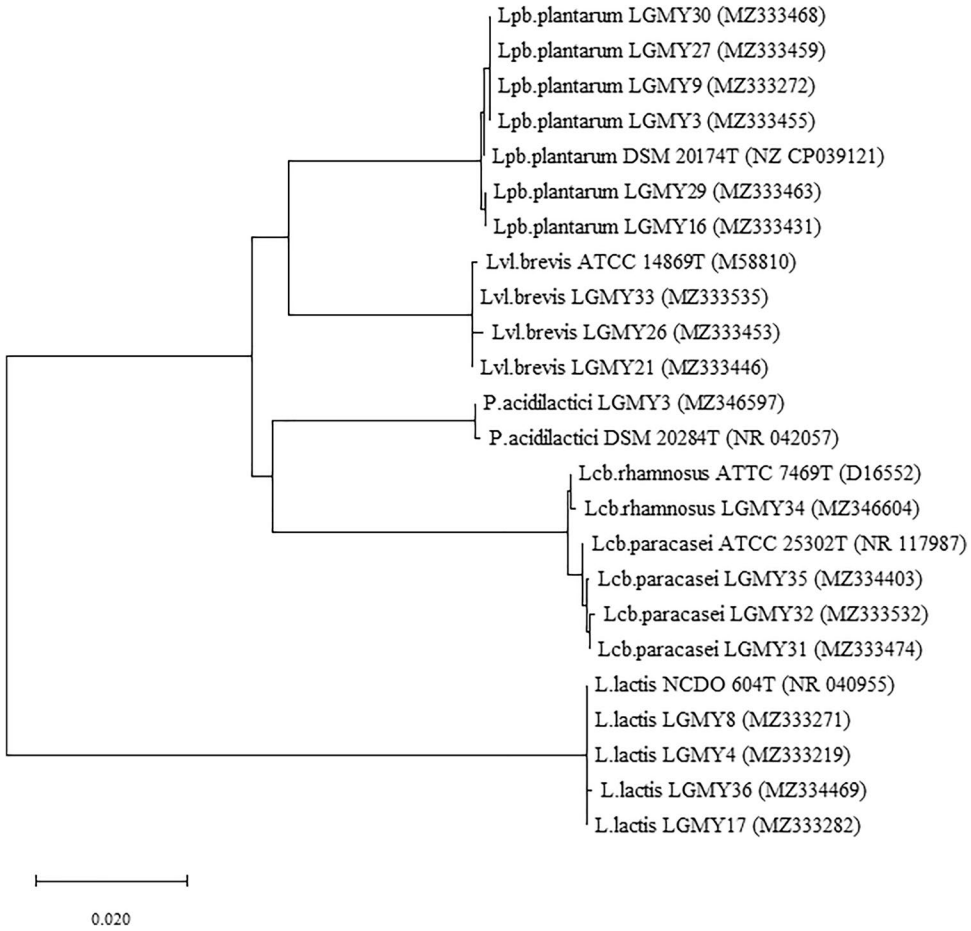

Fig. 2 Phylogenetic tree based on 16S rRNA gene sequences depicting the diversity of *Lpb. plantarum*, *Lcb. paracasei*, *Lvl. brevis*, *Lcb.rhamnosus*, *L. lactis* and *P. acidilactici* isolates by the neighbor-joining method

Proteolytic activity

The results of the proteolytic activity of the strains, determined by the OPA test, are showed in Table 2. *Lcb. paracasei* LGMY31 showed the highest release of amino acids from milk proteins (1.699 mM of leucine equivalents), followed by *Lpb. plantarum* LGMY29 and *Lvl. brevis* LGMY21, which released 0.178 and 0.146 mM of leucine equivalents, respectively. Six other LAB showed a low proteolytic activity, ranging from 0.006 for *Lpb. plantarum* LGMY30 up to 0.092 mM of leucine equivalents for *L. lactis* LGMY8. The remaining strains showed no detectable proteolytic activity.

Exopolysaccharide production

The production of EPS was assessed by evaluating the color of colonies grown on MRS agar containing ruthenium red. All the six strains of *Lpb. plantarum*, *Lcb. paracasei* LGMY31 and LGMY35, and *Lcb. rhamnosus* LGMY34 grew as white colonies, indicating their ability to produce EPS.

Antagonistic activity

The inhibitory potential of the LAB was investigated against four target strains: *L. innocua* CECT 906^T, a surrogate of the human pathogen *L. monocytogenes*; *M. luteus* NCBI 8166 and *Llb. parabuchneri* St2A, two sensitive strains frequently used as target strains: and *L. lactis* subsp. *cremoris* MG1363, a closely related species. Most strains exhibited antagonistic activity against one or more targets in the well-diffusion assay, as shown in Table 2. Nine strains (*L. lactis* LGMY4, LGMY 9 and LGMY36, *Lvl. brevis* LGMY21 and LGMY26, *Lpb. plantarum* LGMY27, *Lcb. paracasei* LGMY31 and LGMY32, *Lcb. rhamnosus* LGMY34) inhibited *Llb. parabuchneri* St2A. Remarkably, only *P. acidilactici* LGMY3 showed specific inhibitory effect against *L. innocua* CECT 906^T. Regarding *M. luteus* NCBI 8166, it was inhibited by *Lpb. plantarum* LGMY29 and LGMY30, *Lvl. brevis* LGMY26, *Lcb. paracasei* LGMY31 and *Lcb. rhamnosus*. Moreover, *L. lactis* subsp. *cremoris* was inhibited by six strains (*L. lactis* LGMY8 and LGMY36, *Lpb. plantarum* LGMY27 and LGMY30, *Lvl. brevis* LGMY33 and *Lcb. paracasei*

Table 2 Technological and functional properties of the isolates

Species	Isolate	Activity in milk[b,c]				Proteolytic activity	EPS production	Antimicrobial activity towards			
		24 h		48 h		OPA[e] (mg Leu released/ml)	Mucoid phenotype	*Llb. parabuchneri* St2A	*L. innocua* CECT 906 T	*M. luteus* NCBI 8166	*L. lactis* subsp. *cremonis* MG1363
		pH	Clotting	pH	Clotting						
P. acidilactici	LGMY3	4.33 ± 00.2	+	4.22 ± 0.10	+ +	0	–	–	+	–	–
L. lactis	LGMY4	4.36 ± 0.03	+	4.13 ± 0.11	+ +	0	–	+	–	–	–
	LGMY8	4.25 ± 0.03	+	4.16 ± 0.10	+ +	0.092	-	-	–	–	+
	LGMY17	4.01 ± 0.02	+	3.73 ± 0.07	+ + +	0	–	–	–	–	–
	LGMY36	3.29 ± 0.13	+	3.05 ± 0.08	+ + +	0	–	+	–	–	+
Lpb. plantarum	LGMY9	3.62 ± 0.05	+ +	3.23 ± 0.099	+ + +	0	+	+	–	–	–
	LGMY16	3.74 ± 0.04	+ +	3.38 ± 0.05	+ + +	0	+	–	–	–	–
	LGMY23	3.16 ± 0.06	+ +	2.96 ± 0.10	+ + +	0	+	–	–	–	+
	LGMY27	3.34 ± 0.11	+ +	3.14 ± 0.11	+ + +[d]	0.027	+	+	–	–	–
	LGMY29	3.55 ± 0.10	+ +	3.02 ± 0.05	+ + +	0.178	+	–	–	+	-
	LGMY30	3.69 ± 0.06	+ +	2.70 ± 0.09	+ + +	0.006	+	–	–	+	+
Lvl. brevis	LGMY21	3.23 ± 0.04	+ +	2.95 ± 0.04	+ + +	0.146	–	+	–	–	–
	LGMY26	4.6 ± 0.08	+ +	4.08 ± 0.08	+ + +	0	–	+	–	+	–
	LGMY33	3.26 ± 0.14	+ +	2.9 ± 0.15	+ + +[d]	0	–	–	–	–	+
Lcb. paracasei	LGMY31	3.62 ± 0.06	+ +	2.65 ± 0.18	+ + +	1.699	+	+	–	+	+
	LGMY32	3.75 ± 0.08	+ +	2.96 ± 0.10	+ + +[d]	0.034	–	+	–	–	–
	LGMY35	3.84 ± 0.11	+ +	2.90 ± 0.08	+ + +	0.008	+	–	–	–	–
Lcb. rhamnosus	LGMY34	4.23 ± 0.08	+	3.29 ± 0.09	+ + +[d]	0.086	+	+	–	+	–

[a]Query coverage of 100% for all the isolates

[b]Inoculated with 10^6 CFU from overnight cultures in UHT milk and incubated at 30 °C

[c]pH of uninoculated milk was 6.6 after 24 and 48 h

[d]Unstable clot

[e]Values are averages from three independent experiments. Standard deviations were < 7%

LGMY31). Overall, *Lcb. paracasei* LGMY31 exhibited the widest range of inhibition against the selected targets.

Production of volatile compounds

The production of volatile compounds differed among strains (Fig. 3A). Ethanol was the main volatile compound produced by all strains, except LGMY8, which probably lack the enzyme alcohol dehydrogenase involved in ethanol production from acetaldehyde (Dan et al. 2019). Acetic acid was also frequently produced (89% of the strains), except for *L. lactis* LGMY4 and *P. acidilactici* LGMY3. Small amounts of 2-propanone were produced by *Lpb. plantarum* LGMY27 and *Lvl. brevis* LGMY21. Acetoine was identified as a minor component in the profile of *P. acidilactici* LGMY3. Almost all the strains produced 3-methyl 1-butanol, except *Lpb. plantarum* LGMY23, *L. lactis* LGMY8 and *Lcb. paracasei* LGMY35, while acetate-3-methyl-1-butanol was produced by *L. lactis* LGMY36.

Furthermore, hierarchical cluster analysis based on the volatile compounds produced by each strain is shown in Fig. 3B. Three main clusters and one outlying group were identified in the dendrogram. The first group include the high producer *L. lactis* LGMY36, the second group includes *Lcb. paracasei* LGMY31 and LMGY35. The third cluster grouped seven medium producers belonging to different species (*Lpb. plantarum* LGMY9, LGMY27, LGMY29, LGMY30, *Lvl. brevis* LGMY21, LGMY26 and *L. lactis* LGMY8). In the fourth cluster, the most numerous with 10 strains, includes the lower producers (*Lcb. paracasei* LGMY32, *Lcb. rhamnosus* LGMY34, *Lpb. plantarum* LGMY16 and LGMY23, *Lvl. brevis* LGMY33, *L. lactis* LGMY4 and LGMY17, and *P. acidilactici* LGMY3). This analysis showed that volatile compounds production is strain-specific and not related to the species. Indeed, different species are equally distributed in these two last groups.

Safety assessment

Antibiotic resistance

All the strains were analyzed for antibiotic susceptibility by disc diffusion method and were classified either as resistant (R), moderately susceptible (MS), or sensitive (S) based on zones of growth inhibition (Table 3). Vancomycin resistance was assayed for the *L. lactis* strains, that resulted all susceptible, since this characteristic is intrinsic for the other LAB species considered, and its evaluation is not required (Aquilina et al. 2012). Most isolates were susceptible to erythromycin (88%), tetracycline (78%), and gentamicin (50%).

Presence of BA-producing genes and capability to produce BA

The genetic potential to synthesize BA was investigated by PCR assays targeting genes responsible for histamine, tyramine and putrescine production, through the HDC, TDC and AGDI pathways, respectively. None of the isolates were PCR positive for the genes *hdc* and *tdc* (Table 3), which indicated the absence of the HDC and the TDC clusters and therefore their inability to produce histamine and tyramine, respectively. However, some isolates were PCR positive for the genes *aguD-aguA* of the AGDI cluster (Table 3). The 700 bp expected amplicon was obtained for the strains *P. acidilactici* LGMY3, *L. lactis* LGMY4, LGMY8, LGMY17 and LGMY36, *Lvl. brevis* LGMY21, LGMY26 and LGMY33. Among those, only *P. acidilactici* LGMY3, and *Lvl. brevis* LGMY21 and LGMY26 were capable to produce putrescine under the examined conditions (Table 3).

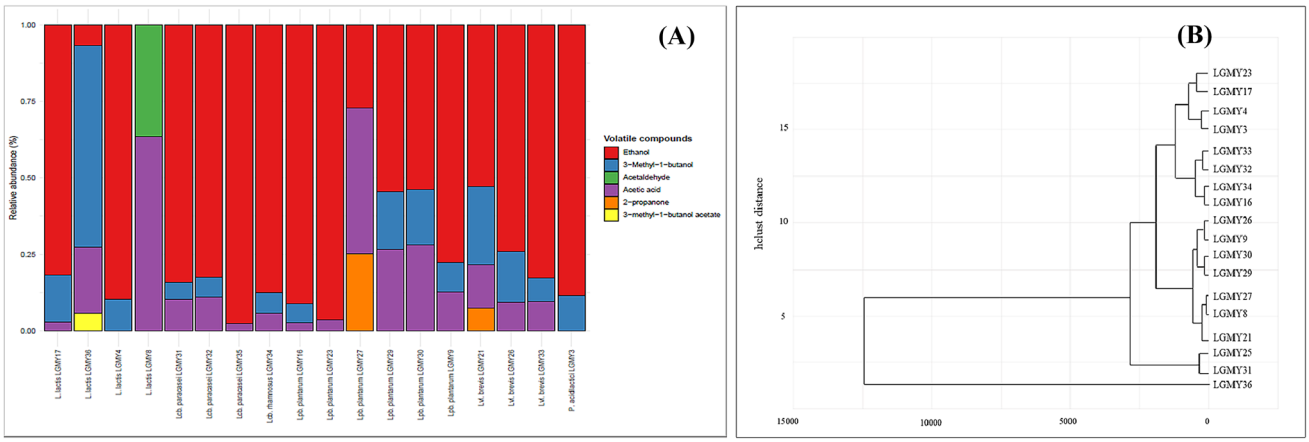

Fig. 3 Relative abundance of volatile compounds produced in milk by the LAB strains (**A**) and hierarchical cluster analysis based on their maximum concentration (**B**)

Table 3 Safety assessment of isolates based on AR phenotypes and BA producing potential

Species	Strain	Antibiotic resistance				PCR			BA production
		Ery	Tet	Gent	Van	HDC	TDC	AGDI	Put
P. acidilactici	LGMY3	S	R	S	n.r	–	–	+	+
L. lactis	LGMY4	R	R	R	S	–	–	+	–
	LGMY8	S	S	S	S	–	–	+	–
	LGMY17	S	S	S	S	–	–	+	–
	LGMY36	S	S	R	S	–	–	+	–
Lpb. plantarum	LGMY9	S	S	S	n.r	–	–	–	n.d
	LGMY16	S	S	R	n.r	–	–	–	n.d
	LGMY23	S	S	R	n.r	–	–	–	n.d
	LGMY27	S	S	R	n.r	–	–	–	n.d
	LGMY29	S	S	MS	n.r	–	–	–	n.d
	LGMY30	S	S	S	n.r	–	–	–	n.d
Lvl. brevis	LGMY21	S	R	S	n.r	–	–	+	+
	LGMY26	S	R	S	n.r	–	–	+	+
	LGMY33	S	S	S	n.r	–	–	+	n.d
Lcb. paracasei	LGMY31	S	S	S	n.r	–	–	–	n.d
	LGMY32	R	S	R	n.r	–	–	–	n.d
	LGMY35	S	S	R	n.r	–	–	–	n.d
Lcb. rhamnosus	LGMY34	S	S	R	n.r	–	–	–	n.d

Ery erythromycin, *Tet* tetracyclin, *Gen* gentamycin, *Van* vancomycin; *S* susceptible, *MS* moderately susceptible, *R* resistant; *HDC* histamine, *TDC* tyramine, *AGDI* agmatine, *Put* putrescine; *n.r* not required; *n.d* not determined

Discussion

The genetic characterization of the LAB strains isolated from several Algerian dairy milks and farm sources allowed their accurate taxonomic assignment at species and strain levels. Particularly, the analysis of $(GTG)_5$ REP-PCR fingerprints highlighted a high genetic biodiversity of the strains. In this research, the lactobacilli showed a remarkable ability to cause rapid milk acidification, which is a desired activity for biotechnological applications (Bintsis 2018). Among the investigated strains, LGM32, LGM34, LGM27 and LGMY33 produced an unstable clot, thus they are not suitable for application in dairy processes. In contrast, *Lpb. plantarum* LGMY23, LGMY29 and LGMY30, *Lvl. brevis* LGMY21 and LGMY33, and *Lcb. paracasei* LGM31, LGM32, LGM35 showed a faster and stronger acidification ability, higher than that reported for others Algerian milk isolates (Bousmaha-marroki and Marroki 2015). This capability is crucial in cheese production, since a rapid acidification favors the coagulation and limits the growth of adventitious undesired microorganisms (Hassaïne et al. 2007).

Regarding antagonistic activity, most of the isolated LAB strains were able to inhibit at least one of the target strains, and, remarkably, *P. acidilactici* LMGY3 inhibited *L. innocua* CECT 906^T. The antimicrobial activity was strain-dependent and could be linked to the production of one or more active compounds during their growth, such as bacteriocins (Gao et al. 2019).

The strains *Lcb. paracasei* LGMY31, *Lpb. plantarum* LGMY29 and *Lvl. brevis* LGMY21 showed interesting results regarding proteolysis. Proteolytic activity is another important characteristic to obtain desirable organoleptic and flavor traits in dairy products (Medjoudj et al. 2020). It was also of interest the capacity of the strains *Lpb. plantarum* LGMY23 and LGMY30 and *Lcb. paracasei* LGM31 and LGM35 to produce EPS and acidify rapidly milk, indicating a good technological potential for application in milk fermentation processes to maximize texture and viscosity (Bachtarzi et al. 2019).

The volatilome was mainly represented by high amounts of ethanol and acetic acid. Homofermentative LAB produce mainly lactic acid from carbohydrates, whereas heterofermentative LAB produce a mixture of lactic acid, acetic acid, ethanol and CO_2 (Widyastuti and Febrisiantosa, 2014). Differently from the other strains, *L. lactis* LGMY8 produced acetaldehyde, which is an important secondary metabolite related to the typical aroma and flavor in yogurts, recognized as "ethereal", "pungent", "fresh" and "green" (Dan et al. 2019). On the other hand, 2-propanone was produced only by the strains *Lpb. plantarum* LGMY27 and *Lvl. brevis* LGMY21. This compound was identified as an odor-active compounds related to wood pulp or hay odor notes (Picon et al. 2019). The related ester 3-methyl-1-butanol acetate

(isoamyl acetate) was exclusively produced by *L. lactis* LGMY36. Esters give an important contribute to the aroma of cheeses and are associated to fruity flavors in Italian and Swiss-type cheeses (Liu et al. 2004).

Regarding the safety assessment, most isolates were resistant to at least one of the antimicrobial compound tested. The major prevalence was to gentamicin (50%); this resistance was previously observed for other LAB isolated from Algerian dairy products (Naceur and Boudjemâa, 2016). The high number of isolates resistant to gentamicin is related to the intrinsic resistance of lactobacilli against this antimicrobial (Campedelli et al. 2019), while the resistance to tetracycline and erythromycin were related to horizontal gene transfer (HGT) events (Anisimova and Yarullina 2018). BA are present in several food products due to the decarboxylase activity of certain strains of LAB (Ladero et al. 2017). The genes related to histamine and tyramine were not detected in any of the strains, while several strains presented genes related to putrescine synthesis via the AGDI pathway. However, putrescine production was confirmed by UHPLC only for *P. acidilactici* LGMY3, and *Lvl. brevis* LGMY21 and LGMY26.

Conclusion

Raw milks and artisanal fermented products represent excellent sources of native LAB strains that can be used as starter or protective cultures. Indeed, the technological and safety aspects evaluated in this research allowed the individuation of some strains that could be good candidates for applications in dairy sector. Particularly, the strains *L. lactis* LGMY8 from cow milk, *Lpb. plantarum* LGMY30 from artisanal cheese and *Lcb. paracasei* LGMY31 from camel milk were safe and unveiled at least one of the desirable technological properties tested, such as high acidification and proteolytic activities in milk, EPS production and antagonistic activity. Further studies will be necessary to evaluate the performances of these strains, singularly or in combination, in artisanal dairy production processes.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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