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Investigating lactic acid bacterial community of Dhan: Insights into the microbiology of western Algerian traditional fermented butter

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Abstract

Background and Objective: Lactic acid bacteria play a fundamental role in the human diet, particularly in fermented foods such as dairy products. This study aimed to investigate microbial diversity of a traditional milk product (Dhan) prepared from raw milk of lactating dairy cows in Northwestern Algeria, highlighting the significance of this traditional dairy product and providing a detailed characterization of its lactic acid bacterial population.

Material and Methods: Nine samples were collected from the available farms in Tiaret and Tissemsilt regions and subsequently cultivated on selective media of MRS agar for *Lactobacillus* and M17 agar for *Lactococcus*. Identification was carried out using API Systems (API 50 CHL) and other biochemical assays.

Results and Conclusion: The bacterial load lactic acid bacteria counts (log10 CFU g^{-1}) averaged 4.92 ±0.47 on MRS and 4.52 ±0.77 on M17 media. Eighteen bacterial isolates were categorized into three genera of *Lactobacillus* (73%), *Lactococcus* (16%), and *Leuconostoc* (11%), with *Lactiplantibacillus plantarum* as the most prevalent species (seven isolates). *Lactobacillus* spp. are the dominant lactic acid bacteria in Dhan and further studies are needed to isolate and characterize biotechnological potentials of these isolated species and their possible roles in innovation.

Keywords: Lactic acid bacteria, traditional Algerian butter, Dhan, microbial diversity, Lactococcus, Lactobacillus, Leuconostoc, fermented foods, food biotechnology, dairy products

What is "already known" on this topic:	➢ Prior to this study, there was only a partial understanding of the role and diversity of lactic acid bacteria in traditional Algerian fermented dairy products. ➢ No previous work had specifically drawn a detailed portrait of the microbial community of Dhan ➢ The identification of dominant and minor species in this product, via classical approaches, filled a notable scientific gap for the sanitary and technological valorization of traditional Algerian butter.
What this article adds:	➢ First comprehensive characterization of lactic acid bacteria diversity in traditional Algerian D'HAN butter. ➢ Lactiplantibacillus plantarum was identified as the predominant species, comprising 39% of isolates. ➢ Six distinct lactic acid bacteria species were isolated from nine artisanal farms, indicating rich microbial biodiversity. ➢ Biochemical profiling reveals the significant biotechnological potential of the isolated strains. ➢ Isolation and identification of 18 bacterial strains from traditional Dhan butter, showcasing potential applications in food and biotechnological industri

1. Introduction

Dairy products, which are vital for human nutrition, are continuously developed worldwide. Since the 1980s, Algerian dairy industry has significantly transformed, moving from a governmental system to a further dynamic private model [1]. However, the quality of raw materials and regulation of the fermentation process are significant problems for the industry and research [2,3].

Traditional fermented milk products play a significant role in diets and cultures of several regions; however, their microbiological diversity is poorly understood, especially in Algeria. Numerous studies have demonstrated that accurate identification of lactic acid bacteria (LAB) in products such as kefir, leben and traditional yogurt is critical for understanding their effects on quality, safety and sensory qualities [4,5].

Traditionally, these microorganisms are identified using polyphasic approach based on culture-dependent methods. This strategy combines the initial isolation of strains on selective media with further phenotypic characterization. This involves assessing key traits such as cell morphology, physiological tolerance to environmental stressors (e.g. temperature and salinity) and biochemical profiles. Assessing metabolic fingerprint of an isolate, particularly its carbohydrate fermentation pattern, is a basis of this methodology for species-level differentiation.

Dhan, a traditional Algerian fermented butter, has received limited attention and its specific microbiology needs further investigations. This microbiological diversity in Dhan deserves further studies because precise identification of the dominant and minor sources of LAB species is essential for controlling sensory and hygienic qualities of the final product [6]. Therefore, the current study aimed to close this gap by characterizing community of LAB in Dhan using physiological and biochemical methods to preserve and improve the quality of this product.

2. Materials and Methods

2.1. Sampling Region

Samples were collected from Tissemsilt (35° 36′ N, 1° 49′ E) and Tiaret (35° 22′ N, 1°19′ E), Algeria. These regions included Mediterranean climates with elevation of approximately 1,080 m above the sea level, average annual rainfall of 300–500 mm and average annual temperature of 22 °C. During the sampling time from February to May 2023, the ambient temperature varied 14–28 °C (Figure 1).

2.2. Sample Collection

Purposive sampling approach was used to identify authentic producers of traditional Dhan butter across the Tiaret and Tissemsilt regions. Stringent selection criteria were established, limiting eligibility to registered dairy farms with (i) active traditional production, (ii) verified compliance with national hygiene standards and (iii) official state accreditation. This rigorous process yielded a total of nine qualifying farms—four in Tiaret and five in Tissemsilt—representing all the accredited producers in the study area. This strategy ensured that microbiological analysis was carried out on authentic standardized samples. All samples were aseptically collected from the nine farms and processed at the Laboratory of Microbiology, University of Tissemsilt, Algeria.

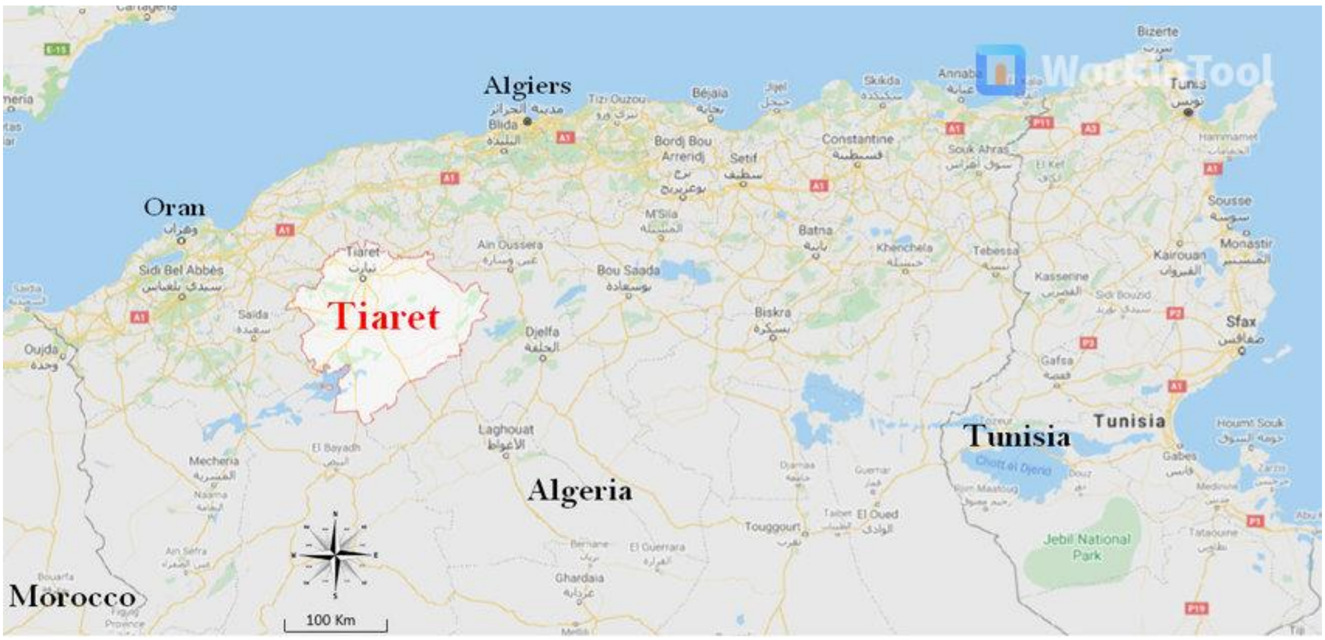

Figure 1. Sampling area of Tissemsilt and Tiaret in Algeria

Lactic Acid Bacteria Isolation and Enumeration

All microbiological chemicals and culture media used in this study were purchased from Lab Quality, Algeria. Samples were diluted in sterile 0.9% NaCl solution and spread-plated onto M17 and MRS agars for *Lactococcus* and *Lactobacillus* spp. The pH of the MRS agar was adjusted to 5.4 to enhance recovery. Plates were incubated for 24–72 h and colonies with typical LAB morphology were selected, counted and purified [7].

2.3. Culture Maintenance and Purification

The LAB isolates were purified via alternating subcultures on MRS agar and broth. Purified isolates were stored at -20 °C in MRS broth supplemented with 30% (v/v) sterile glycerol.

2.4. Characterization and Identification of the Lactic Acid Bacterial Isolates

2.4.1. Morphological and Basic Biochemical Characterization:

Colony characteristics, cell morphology, Gram reaction, catalase activity and oxidase activity assessments were carried out on agar plates based on the standard protocols [7].

Physiological Characterisation

Gas Production from Glucose: Isolates were inoculated into MRS broth (citrate-free) with inverted Durham tubes and incubated at 30 °C for 24–48 h. Gas accumulation indicated CO_2 production (heterofermentative).

Arginine Dihydrolase (ADH) Activity: Arginine dihydrolase (ADH) activity was assessed using M16BCP media incubated anaerobically (30 °C, 72 h). A purple-to-yellow color change revealed ADH positivity.

Growth under Stress Conditions: Growth was monitored by measuring OD_{600} (UV-visible 7305 spectrophotometer, Labo and Co, France) against uninoculated controls after incubation under specific conditions. The NaCl tolerance was assessed using MRS/M17 broth with 4 and 6.5% (w/v) NaCl at 37 °C for 24–48 h. Temperature tolerance was Assessed in MRS/M17 broth at 10, 15, 37, 40 and 45 °C for 24–72 h [8].

2.4.2. Assessment of Sugar Fermentation Profiles

Cell pellets were achieved from 18-h cultures grown at 30 °C by centrifugation (5000× g, 5 min). The washed pellets were resuspended in indicator media (MRS BCP for *Lactobacillus* spp. and 0.5% peptone water with bromothymol blue for *Lactococcus* spp.) containing 0.5% of a sugar. Tubes were overlaid with sterile paraffin oil and incubated at 30 °C for 3–7 d). The color change indicated fermentation. Sugars included xylose, maltose, galactose, D-sorbitol, arabinose, mannitol, L-rhamnose, sucrose, lactose, D-fructose, glucose and esculin.

2.5. Carbohydrate Fermentation Profile

Eighteen isolates were assessed using API 50 CHL strips, according to the manufacturer's instructions. This standardized identification system has been reported as an important reliable tool for lactobacilli identification based on phenotypical characteristics [9]. Inocula were prepared from fresh colonies (MRS agar; 30 °C, 48 h) suspended in API 50 CHL media. Strips were inoculated (100 µl per well), overlaid with sterile mineral oil and incubated at 30 °C for 48 h under humid conditions. Color changes were recorded after 24 and 48 h and results were interpreted using APIweb software.

2.6. Quality Control

Several quality control assessments were used to ensure validity of the results. Sterility of all culture media and diluents was verified by incubating uninoculated negative controls (uninoculated media) within the experimental samples, ensuring aseptic integrity of the procedure. To validate accuracy of the biochemical assays, resulting profiles of the identified species were systematically compared with those in the scientific literature and Bergey's Manual of Systematic Bacteriology. This comparative analysis verified that the observed reactions (e.g. Gram staining, catalase test and carbohydrate fermentation pattern) were similar to the expected profiles for the identified taxa.

2.7. Data analysis

Statistical analysis was carried out using R software. For each sample, triplicate assessments were averaged. Overall means and 95% confidence intervals were calculated from nine sample means using Student's t-distribution.

3. Results and Discussion

3.1. Bacterial Counts and Isolation

The LAB counts (log10 CFU g^{-1}) averaged 4.92 ±0.47 on MRS and 4.52 ±0.77 on M17 media. The higher variability observed on M17 media (CV = 17%) than on MRS (CV = 9.6%) reflected the selective nature of these media for various LAB groups. These counts were smaller than those reported in other studies. A Turkish study on yak butter [9] reported greater levels of *Streptococcus thermophilus* (5.42–6.30 log CFU ml^{-1}) and *L. delbrueckii* subsp. *bulgaricus* (5.20–5.80 log CFU ml^{-1}) in goat, ewe and cow milk butters. Furthermore, the present results were smaller than those of an Ethiopian study [7], which reported *Lactobacillus* counts ranging from 4.5×10^7 to 2.3×10^8 CFU ml^{-1} and *Lactococcus* counts ranging from 1.12×10^7 to 2.75×10^9 CFU ml^{-1} in raw milk, cheese and yogurt.

3.2. Microbiological Quality Assessment

The microbiological quality parameters of traditional butter (Dhan) samples were assessed to report the overall hygiene of the product (Table 1). All samples were analyzed in triplicate to ensure reproducibility. The total aerobic mesophilic flora (FMAT) averaged 2.04 ±0.27 × 10^4 CFU g^{-1} [95% CI, 1.83–2.24], indicating a moderate microbial load typical of the traditional fermented dairy products. The total coliform (1.37 ±0.16 × 10^3 CFU g^{-1}) and fecal coliform (1.45 ±0.23 × 10^2 CFU g^{-1}) counts were included in the acceptable ranges for the products. Yeast and mold populations included 2.47 ±0.26 × 10^3 CFU g^{-1} and 1.13 ±0.14 × 10^3 CFU g^{-1}, respectively. The low coefficients of variation (< 15%) between the replicates demonstrated good analytical precisions.

3.3. Morphological and Phenotypical Characterizations

Of the 18 isolates from traditional Dhan samples, 73% were identified as Lactobacillus, 11% as Lactococcus and 16% as Leuconostoc spp. (Figure 2). On MRS media, Lactobacillus colonies were rounded, smooth and convex with whitish to beige in color. On M17 media, Lactococcus isolates formed white to yellowish circular colonies and showed diplococcal cell arrangement in chains and clusters.

All the isolates were Gram-positive (Figure 3). Thirteen isolates showed rod morphology (LBS1a through LBS1m) characteristics of *Lactobacillus*, whereas five isolates showed cocci morphology characteristics, typical of *Lactococcus* and *Leuconostoc* spp.

Table 1. The microbiological quality parameters of traditional butter samples. (mean ± SD [95% CI])

Sample	Total aerobic mesophilic flora (UFC x 10^4/g)	Total Coliformes (UFC x 10^3/g)	fecal coliform (UFC x 10^2/g)	Yeast (UFC x 10^3/g)	mold (UFC x 10^3/g)
1	1.78 ± 0.59	1.27 ± 0.09	1.53 ± 0.06	2.51 ± 0.08	1.00 ± 0.04
2	1.82 ± 0.17	1.62 ± 0.22	1.20 ± 0.10	2.79 ± 0.26	1.20 ± 0.12
3	2.43 ± 0.09	1.51 ± 0.23	1.80 ± 0.10	2.09 ± 0.10	0.94 ± 0.04
4	2.26 ± 0.30	1.36 ± 0.07	1.43 ± 0.06	2.67 ± 0.09	1.13 ± 0.04
5	1.73 ± 0.18	1.41 ± 0.04	1.23 ± 0.06	2.58 ± 0.10	1.25 ± 0.05
6	2.10 ± 0.08	1.11 ± 0.09	1.53 ± 0.06	2.10 ± 0.08	0.96 ± 0.04
7	2.30 ± 0.26	1.54 ± 0.11	1.70 ± 0.10	2.75 ± 0.13	1.33 ± 0.06
8	2.16 ± 0.42	1.32 ± 0.08	1.50 ± 0.10	2.43 ± 0.14	1.21 ± 0.07
9	1.75 ± 0.06	1.21 ± 0.01	1.10 ± 0.00	2.32 ± 0.03	1.11 ± 0.01
Total	2.04 ± 0.27 [1.83 - 2.24]	1.37 ± 0.16 [1.25 - 1.50]	1.45 ± 0.23 [1.27 - 1.63]	2.47 ± 0.26 [2.27 - 2.67]	1.13 ± 0.14 [1.02 - 1.23]

(a) (b)

Figure 2. Morphological characteristics of isolates on MRS (a) and M17 (b) media

Figure 3. Gram staining of Lactic Acid Bacteria Isolates (LAB)

3.4. Species Identification

Species identification revealed the following distribution of *Lactiplantibacillus plantarum* (*n* = 7, 39%), *Lacticaseibacillus casei* (*n* = 3, 17%), *L. acidophilus* (*n* = 3, 17%), *L. cremoris* (*n* = 2, 11%), *L. lactis* (*n* = 2, 11%) and *L. mesenteroides* subsp. *dextranicum* (*n* = 1, 6%).

3.5. Physiological and Biochemical Characteristics

Results achieved for the six isolates verified their identification as *L. plantarum*. Their phenotypic characteristics were consistent with published data. The observed fundamental characteristics such as Gram-positive staining, absence of catalase activity and facultative heterofermentative metabolism were classic markers of *L. plantarum*, which has widely been documented in studies on isolates from diverse food sources [8,10,11]. Growth of strains at 10 °C and their inhibition at 45 °C verified their mesophilic nature. This characteristic was shared by *L. plantarum* strains from fermented dairy and meat products, indicating their adaptation to moderate fermentation conditions [10,11]. Moreover, their tolerance to 4% not to 6.5% NaCl was similar to that of many strains used in processes where salt played a preservative role [8,11].

Carbohydrate fermentation profile provided a distinctive biochemical signature for these isolates. Their ability to metabolize a wide range of sugars, including maltose, galactose, sorbitol, mannitol and lactose, reflected high metabolic versatility that characterized *L. plantarum* and explained its prevalence in diverse food ecosystems [8,11]. This flexibility was a major technological asset for its use as a starter culture. The role of dominant Dhan strains in microbial stabilization of the product was supported by the associations between increased pathogen inhibition and presence of plnEF and plnJ genes in *L. plantarum* from fermented products such as Ishizuchi-kurocha tea [12].

Strains S1a, S1h and S1i, identified as *L. casei*, showed characteristics similar to this species; however divergences were reported. Gram-positive staining and negative reaction to catalase were classic markers for *Lactobacillus* spp. and *L. casei*, particularly [9]. Temperature tolerance was similar to that of literature with growth observed at 10 and 40 °C but not at 45 °C. This was similar to the reported optimal growth temperature of 41–42 °C and inhibition at higher temperatures [13]. Similarly, presence of growth at 4% NaCl and absence of growth at 6.5% NaCl were similar to salt tolerance profile described for *L. casei* [14]. Fermentation profiles of certain sugars such as glucose, fructose, galactose and mannitol as well as hydrolysis of esculin were similar to the expected biochemical profiles [15].

However, results revealed significant differences from the typical profiles described in the literature. The most important difference included inability of the assessed strains to ferment either lactose or sucrose. The *L. casei* is a bacterium widely used in the fermented dairy products because of its effective ability to metabolize lactose [16]. Numerous studies have reported its ability to use sucrose [17]. Absence of fermentation of these two sugars was therefore atypical and could indicate strain-specific characteristics of the isolates since sugar metabolism could be strain-dependent and presence of specific genes often located on plasmids [18]. Additionally, gas production by these isolates suggested heterofermentative metabolism. This was in contrast to typical behavior of *L. casei*, which was facultatively homofermental and produced primarily lactic acid from hexoses without gas formation [19].

Analysis of the results for S1k, S1l and S1m strains, identified as *L. acidophilus*, demonstrated similarity to the phenotypical and biochemical characteristics of this species, verifying accuracy of the identification [15]. The observed fundamental features such as Gram-positive staining and negative reaction to catalase were classic markers of LAB [10,20]. Strict homofermentative metabolism indicated by the absence of CO_2 production from glucose was a distinctive characteristic of *L. acidophilus* [10]. These basic assays strongly indicated that these isolates belonged to the homofermentative *Lactobacillus* group.

Growth profiles were investigated under various temperatures and salinity conditions. Growth at 40 not 10 and 45 °C verified mesophilic characteristic of the strain, whose optimal temperature range was generally between 37 and 42 °C. This thermal profile was in contrast to that of other LAB such as the psychrotrophic species of *L. piscium* adapted to cold temperatures and lacked growth at temperatures greater than 29 °C [20]. Similarly, tolerance to 4% not 6.5% NaCl was similar to physiological characteristics of many *L. acidophilus* strains. However, this tolerance might vary. Indeed, studies have isolated strains of *Lactobacillus* capable of growing at 6.5% NaCl, highlighting intraspecific variations associated to the adaptation of the strain to its environment [10].

Fermentation profile of sugars is the most robust element for the microbial verification. Ability of the strains to metabolize a wide range of carbohydrates, including glucose, fructose, galactose, lactose, sucrose and maltose, is typical for *L. acidophilus*, a species commonly isolated from fermented dairy products [10]. Positive fermentation of D-sorbitol and mannitol although showing strain-to-strain variations is a documented characteristic of the reference strain *L. acidophilus* ATCC 4356, supporting accuracy of the identification. In contrast, *L. acidophilus* strains from Ethiopian fermented milk lacked this fermentation ability, demonstrating metabolic diversity within the species [10]. Furthermore, hydrolysis of aesculin and inability to ferment pentoses such as xylose and arabinose completed the characteristic biochemical profile. However, fermentation of xylose has been observed in wild strains [10]

Table 2. Physiological and biochemical characteristics of *Lactobacillus* and cocci strains isolated from Dhan

	Groupe A						Groupe B				Groupe C			Groupe D		Groupe E	Groupe F	
Souche LB	S1a	S1b	S1c	S1d	S1e	S1f	S1g	S1h	S1i	S1j	S1k	S1l	S1m	LN S3a	LN S3b	LN S3c	LC S2a	LC S2b
Gram	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CO_2	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het	+ Het
A.D.H.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Température																		
10°C	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
40°C								+	+	+	+	+	+	-	-	-	-	-
45°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.5% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sugar Profile																		
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Maltose	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-sorbitol	+	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
L-rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculine	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Identification	*Lactiplantibacillus plantarum*						*Lacticaseibacillus casei*				*Lactobacillus acidophilus*			*Leuconostoc lactis*		*Leuconostoc mesenteroides* subsp. *Dextranicum*	*Lactococcus Cremoris*	

Analysis of the results for the LNS3a and LNS3b strains identified as *L. lactis* demonstrated characteristics similar to the typical characteristics of this species, as described in the scientific literature. Isolates demonstrated typical LAB characteristics, including Gram-positive staining and negative catalase reaction. Production of gas verified strict heterofermentative metabolism of *L. lactis*, a distinctive characteristic of the genus. This metabolism, via the phosphoketolase pathway, converted hexoses to lactic acid, ethanol and CO_2 [22].

Temperature tolerance profile was similar to the species profile. Ability to grow at 10 °C while inhibited at 40 and 45 °C corresponded to the mesophilic and psychrotrophic characteristics of the species. Indeed, *Leuconostoc* spp. involved in food fermentation include optimal growth temperature generally between 18 and 22 °C, with inability to grow at higher temperatures [21]. The strain grew at 4% not 6.5% NaCl demonstrated salt tolerance similar to that of the species profile. Many *Leuconostoc* spp. Include this particular tolerance, allowing them to outcompete less tolerant microorganisms in moderately saline environments such as vegetable fermentations [21]. Fermentation profile of sugars supported this identification.

Ability to metabolize monosaccharides (glucose, fructose, galactose) and disaccharides (lactose, sucrose, maltose) is a typical characteristic of *L. lactis*, a bacterium widely used in dairy industries and plant fermentations [22]. Inability to ferment mannitol is a significant point. Although these strains ferment fructose, they do not convert it into mannitol as an ability of other heterofermentative species of *Leuconostoc* [23]. Absence of pentose fermentation (xylose, arabinose) and esculin hydrolysis completed the biochemical profile. Characteristics of strain LNS3c were similar to those described for *L. mesenteroides* subsp. *dextranicum* in the scientific literature. The isolate demonstrated typical *Leuconostoc* characteristics, including Gram-positive staining, negative catalase reaction and strict heterofermentative metabolism [24].

Growth profile was similar to that in literatures. Ability to grow at 10 °C not 40 °C verified mesophilic and psychrotrophic characteristics of the species, whose optimal temperature is generally between 25 and 30 °C [25]. Similarly, tolerance to 4% not 6.5% NaCl was similar to the reported characteristics, as growth is typically inhibited at concentrations greater than 6% [26]. Fermentation profiles of sugars supported this identification. Ability to metabolize glucose, fructose, galactose and lactose is a typical characteristic of this species [27]. The positive fermentation of sucrose is particularly significant because *L. mesenteroides* subsp. *dextranicum* is renowned for the use of this sugar to produce extracellular polysaccharides, particularly dextran [25]. Inability to ferment maltose was a distinctive result, although strain-specific variation in sugar metabolism has been reported [27].

Analysis of the results for LCS2a and LCS2b strains identified as *L. cremoris* showed excellent similarity to the typical characteristics of this species. Isolates demonstrated typical *Lactococcus* characteristics, including Gram-positive staining and negative catalase reaction. Furthermore, absence of gas production (CO_2) verified their strict homofermentative metabolism, converting sugars to lactic acid primarily [10].

Temperature and salt tolerances were similar to those reported in literatures. Growth observed at 10 not 40 °C was a typical characteristic of mesophilic bacteria such as *L. cremoris*, whose optimal temperature is nearly 30 °C and growth is generally inhibited at temperatures nearly 40 °C [10,11]. The 4% NaCl tolerance and 6.5% inhibition characteristics were similar to the reported characteristics, as the viability of *L. cremoris* decreases significantly with increasing salt concentration with numerous strains inhibited by concentrations greater than 5% [28,29]. Fermentation profiles of the sugars supported this identification. Ability to metabolize a wide range of carbohydrates, including glucose, lactose, galactose, sucrose and maltose, was a well-documented characteristic of *L. cremoris*, a species widely used as a starter culture in the dairy industry [30]. Xylose fermentation, though less consistently reported, is strain-dependent.

In this study, several methodological limitations must be acknowledged. First, the relatively small sample size due to the practical constraints meant that while the present findings provided valuable preliminary insights, larger-scale studies are still necessary. Second, this study relied on biochemical and phenotypic methods for species identification. This approach was chosen to establish a functional profile of the Dhan microbiota and provide a metabolic fingerprint of the isolates. While this method provides valid preliminary identification for the study primary aim, the current authors recognize that the absence of molecular verification is a key limitation. Molecular techniques such as 16S rRNA gene sequencing were not available due to practical constraints. Therefore, these isolates represent prime candidates for further genetic verifications to validate their taxonomy. Such molecular approaches can provide higher resolution identifications and further investigate potentials of this unique microbiota.

4. Conclusion

This study highlighted diversity and abundance of LAB isolated from Dhan of raw milk in western Algeria. Six LAB species were identified using traditional biochemical methods with *L. plantarum* showing unique predominance. This finding represented the first report of LAB diversity in Dhan, revealing unique microbial biodiversity of Dhan microbiota and broadening current understanding of the microbial biodiversity of traditional fermented milk products. Physiological and biochemical characteristics demonstrated by the isolated strains suggested that they warrant further investigations for potential technological and probiotic uses in food industries. These findings contributed to the present understanding of traditional fermented products as sources of beneficial microorganisms with potential uses in food preservation and quality improvement.

5. Acknowledgements

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6. Declaration of competing interest

The authors declare no conflict of interest.

7. Authors' Contributions

Silarbi tayeb : Methodology designing, data curating, data analyzing and writing original draft of the manuscript; laabas saadia: conceptualizing, methodology designing, supervising and reviewing and editing the manuscript; chahbar mohamed: methodology designing, supervising and reviewing and editing the manuscript; Khaled hamden: supervising and reviewing the manuscript. All authors read and approved the final version of the manuscript.

8. Using Artificial Intelligent Chatbots

No chatbots or artificial intelligence tools were used for data analysis, scientific content generation, or interpretation of results in this research.

9. Ethical Consideration

All experimental procedures were carried out in accordance with national hygiene and ethical standards. No animal samples were taken without the consent of producers. The study protocol did not require the collection of information from people or live animals, and was limited to the collection and analysis of dairy products. The confidentiality of participating producers has been preserved.

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بررسی جامعه باکتری اسید لاکتیک دحان (Dhan): بینش‌هایی در مورد میکروب‌شناسی کره تخمیری سنتی غرب الجزایر

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چکیده

سابقه و هدف: باکتری‌های اسید لاکتیک نقش کلیدی در رژیم غذایی انسان، به‌ویژه در محصولات لبنی تخمیری دارند. این پژوهش با هدف بررسی تنوع میکروبی کره سنتی دحان، که از شیر خام گاوهای شیرده در شمال‌غرب الجزایر تهیه می‌شود، انجام شد تا اهمیت این محصول سنتی و ویژگی‌های جمعیت باکتری‌های اسید لاکتیک آن را مشخص کند.

مواد و روش ها: نه نمونه از مزارع مناطق تیارت و تیسمسیلت جمع‌آوری و در محیط‌های کشت انتخابی MRS (برای لاکتوباسیلوس) و M17 (برای لاکتوکوکوس) کشت داده شد. شناسایی سویه‌ها با استفاده از سیستم API 50 CHL و آزمایش‌های بیوشیمیایی انجام گرفت .

یافته‌ها و نتیجه‌گیری: میانگین بار باکتریایی اسید لاکتیک ($\log 10$ CFU g^{-1}) در محیط MRS برابر ۴،۹۲ ± 0.47و در محیط M17 برابر ۴،۵۲ ± ۰،۷۷ بود. از ۱۸ سویه جداسازی‌شده، سه جنس شناسایی شد: لاکتوباسیلوس (۷۳٪)، لاکتوکوکوس (۱۶٪) و لوکونوستوک (۱۱%)، که گونه *Lactiplantibacillus plantarum* با هفت سویه غالب بود .لاکتوباسیلوس‌ها به‌عنوان باکتری‌های اصلی اسید لاکتیک در دحان شناخته شدند. این مطالعه نشان داد که دحان دارای تنوع میکروبی غنی است و سویه‌های شناسایی‌شده پتانسیل بالایی برای کاربردهای بیوتکنولوژیکی دارند. تحقیقات بیشتر برای بهره‌برداری از این سویه‌ها در نوآوری‌های غذایی ضروری است.

واژگان کلیدی: : باکتری‌های اسید لاکتیک، کره سنتی الجزایری، دحان، تنوع میکروبی، لاکتوکوکوس، لاکتوباسیلوس، لوکونوستوک، غذاهای تخمیری، بیوتکنولوژی غذایی، محصولات لبنی

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