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Exploration of the biodiversity of the lactic flora of a traditional Algerian cheese (type: *Jben*) from the steppe zones of the Algerian center of Djelfa.

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Abstract – Five samples of traditional Algerian cheese (type: *Jben*) collected from five different sites in the Algerian center of Djelfa's steppe zones, were studied by determining its physicochemical and microbiological characteristics. The diversity and density of the lactic acid bacteria of this cheese have been the subject of phenotypic, physiological, and biochemical analyses. The average pH of the samples was 4.39 ± 0.15, the average titratable acidity value was 69.4 ± 3.11 °D and the results of the microbiological analyses were; total aerobic mesophilic flora 6.34 x 10^6 CFU/g, coliform bacteria 1.44 x 10^4 CFU/g, Yeasts 1.88 x 10^4 CFU/g, *Staphylococcus*, *Salmonella*, and Molds were not detected. Among the lactic microflora studied, 164 strains of lactic acid bacteria were isolated and purified. This study revealed the presence of 101 Cocci-shaped and 63 rod-shaped. The Coccids are represented by the *Enterococcus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus*; the genus *Lactobacillus* is detected and identified in varying proportions. This diversity could be developed to have an interest in the cheese manufacturing of the *Jben* type, particularly in its valorization, and thus could be used as a probiotic indication for health.

Keywords: Exploration, Biodiversity, Lactic Flora, Jben, Steppe Zones.

I. INTRODUCTION

Milk's microorganisms have long been employed in milk processing and preservation. People have been consuming fermented milk since prehistoric times. A wide variety of cheeses may be produced because of the microbial flora found in milk and the action of rennet. Because of this, the natural flora of milk which was initially the primary cause of its degradation has been sought after for its acidifying, lipolytic, proteolytic, and aromatic qualities through specialized knowledge passed down through the generations. **[1]**. These dairy microbial agents are vital to the production of many different types of cheeses because of their proteolytic, lipolytic, texturizing, flavorful, and antibacterial qualities. Studies on the microbiology of cheeses have revealed that these foods are actually authentic microbial ecosystems, with a multitude of interactions occurring both between the individual bacteria and with their local surroundings. Thus, certain bacteria proliferate actively at a particular stage of maturity whereas others tend to vanish, depending on the physicochemical parameters of the medium. As a result, the ratios of significance among the various groups of microorganisms and their balances are ever-changing. **[2]**. The traditional

family-level method of producing cheese is still in use in Algeria. It is vital to understand that the natural microflora of *Jben* produces its pleasant organoleptic features and nutritional qualities, which have led to a surge in consumption of this highly valued traditional variety in steppe areas. The production process is still in use today. A multitude of research using various technologies has established the primary importance of these bacteria in determining the sensory attributes of cheeses. The cheese becomes less flavorful and has less variation in scent when the bacteria in raw milk are eliminated (via microfiltration and pasteurization) **[3]**. Beyond the sensory interest that certain microbial populations of cheeses can bring, their inhibitory effect against pathogenic microorganisms has also been demonstrated **[4],[5]**. Studies of this type are rare concerning the microflora of traditional cheeses made by hand in Algeria. This work therefore proposes: To explore the biodiversity of the lactic microflora of a traditional *Jben* cheese resulting from the artisanal manufacture of the steppe regions of Djelfa. This biodiversity of the lactic microflora will be studied by different identification techniques according to different levels of specificity.

II. MATERIAL AND METHODS

In our experience, we used biological material: Fresh cheese of the *Jben* type: cheese samples were taken aseptically in sterile containers from 05 different sites in the cities of Birine (B), Ain Oussera (O), Djelfa (D), Ain l'bel (A) and Massaad (M) in the Wilaya of Djelfa and kept at low temperature until the start of experimental laboratory analyzes.

Sampling area: The middle region of northern Algeria is home to the Wilaya of Djelfa (Figure 1). The wilaya can continue to grow because of its centrality. It is an unquestionable crossing point and the ideal connection between the country's east and west as well as its north and south. The Wilaya of Djelfa is mostly a pastoral sheep farming region in Algeria due to the characteristics of its natural environment and the size of its land. **[6]**.

Fig.1 Location of the sampling area (https://www.google.com/).

1. Physicochemical analysis: All of the traditional *Jben* cheese samples under study were collected, sent straight to the laboratory in chilled containers (four degrees Celsius), and examined right away without being kept in storage. The pH of the cheese was measured using a conventional protocol **[7]**. The samples' acidity was evaluated in triplicate, and the average results were given **[8]**. Five drops of phenolphthalein are added to a 1% indicator in a tiny beaker containing 10 milliliters of sample. Use 0.1 N NaOH to titrate the sample. Keep in mind that the sample ought to be faintly pink **[9]**.

2. Microbiological analysis:

2.1. Determination of microbiological quality: The total aerobic mesophilic flora (TAMF) was isolated and counted using conventional microbiological techniques and medium, with decimal dilutions in physiological water. A sterile dropper was used to 1 ml of each dilution into each of the three Petri dishes. The Petri dishes were melted, cooled to between 40 and 45 °C, and then stirred before being incubated for a full day at 37 °C. The product's average CFU/g was assessed **[10]**. Furthermore, the technique of counting colonies to determine the quantity of coliform bacteria was applied. One milliliter is divided between two Petri plates using the decimal dilutions. VRB mold agar, which has been melted and chilled

to 45 °C, contains agar and lactose, bile salts, purple crystal, and an indicator in each box. The above-prepared boxes are incubated for a full day at 35 °C. Following incubation, the reddish-purple colonies are tallied, and the following formula is used to determine the average value: $\mathbf{N = \Sigma c/ (n_1 + 0.1\ n_2) *d}$, (*c: is the sum of the counted colonies; n_1 is the number of cans to be counted from the first dilution; n_2 is the number of cans to be counted from the second dilution; d is the dilution ratio corresponding to the first dilution used*) **[10]**. In addition to adding egg yolk and tellurite to Baird-Parker Agar, the species of pathogenic and opportunistic bacteria, including S*taphylococcus* and coagulase-positive *Staphylococcus*, were enumerated. For a duration of 24 to 48 hours, the dishes were incubated at 37 °C. The dubious colonies were counted after they had grown. The colonies are divided into two categories: atypical (jet black to dark gray colonies, full margin without halo) and normal (smooth, convex, opaque zone throughout, entire border with a bright halo beyond it). **[11]**. To identify *Salmonella*, 225 milliliters of buffered peptone water were used to suspend 25 grams of cheese, which was then pre-enriched and incubated for 24 hours at 37 degrees Celsius. 10 ml of tetrathionate broth (selective enrichment) was added to 1 ml of pre-enrichment broth, and the mixture was incubated for 24 hours at 42 °C. Brilliant green agar (selected medium) and *Salmonella* and *Shigella* agar (SSA) were streaked with a full loop of tetrathionate broth, and the mixture was incubated for 24 hours at 37 °C. **[12]**. 10 g were extracted aseptically and homogenized for 5 minutes at a normal speed in 50 ml of 1% sterile peptone water to determine the quantity of molds and yeasts. **[13]**. Samples were spread out on Potato Dextrose Agar (PDA) after being diluted in series. After the agar has gelled, invert the flat box and incubate at 28 °C±1 °C for five days. If required, use a magnifying lens to see or identify the yeasts and molds that correlate to the various dilution rates. expressed as colony-forming units, or CFUs. Choose the box containing counts ranging from 10 to 150 CFU, then count the molds and yeasts, accordingly, based on how they seem. **[14]**. Using M17 and MRS agar at pH 6.2 and an incubation period of 24 to 48 hours, the lactic acid bacteria were counted. The techniques outlined by the International Dairy Federation (IDF) for the different LABs stated in Table 1 were followed to selectively isolate the lactic acid bacteria through medium culture. **[15]**.

Table 1. Media used and incubation conditions for the isolation of LAB.

Micro-organisms	Isolation media	T (°C)	Duration (h)	Incubation
Lactic enterococci	**M17 [16]**	**45**	**72**	**Aerobiosis**
Lactococci	**Elliker [17]**	**30**	**72**	**Aerobiosis**
Leuconostocs	**Hypersaccharose [18]**	**25**	**72-144**	**Aerobiosis**
Pediococci	**M17 [16]**	**30**	**72**	**Aerobiosis**
Mesophilic lactobacilli	**MRS [19]**	**30**	**24-36**	**Anaerobiosis**
Thermophilic lactobacilli	**MRS [19]**	**45**	**24-36**	**Anaerobiosis**

T (°C): optimal growth temperature.

2.2. Exploration of the biodiversity of the lactic microflora:

2.2.1. Determining the isolates of lactic acid bacteria (LAB): It was done at the University of Mostaganem in Algeria's Laboratory of Sciences and Techniques of Animal Production (LSTAP), using

phenotypic methodologies. There were two processes involved in the genus stage identification of LAB isolates. The first involves creating catalase, Gram staining, and spore development to test each isolate. The second is predicated on the kind of fermentation and morphological analysis (both macroscopic and microscopic). The isolates that were typical of the dominating flora were identified using the following identification criteria:

- For *Lactobacillus* **[20], [21]**.
- For *Streptococcus, Enterococcus,* and *Lactococcus* **[22], [23]**.
- For *Leuconostoc* and *Pediococcus* **[24], [25]**.

2.2.2. The physiological and biochemical criteria: they are based on the following tests:

- The Sherman test and the thermoresistance at 60.5 °C. for 30 min **[23]**.
- Growth, on M17 and MRS medium, was monitored for temperatures of 5 °C, 10 °C and 15 °C after incubation for 5-7 days and 30 °C, 40 °C, 45 °C and 50 °C after incubation for 24 to 48 hours. Depending on the temperatures, the isolates of the genera were tested as follows: *Leuconostoc* at 5 °C, *Lactococcus* at 10 °C, *Lactobacillus* and *Leuconostoc* at 15 °C, *Leuconostoc* at 37 °C, *Pediococcus, Enterococcus, Streptococcus* and *Lactococcus* at 40 °C, *Enterococcus, Lactococcus, Lactobacillus, Pediococcus* and *Leuconostoc* at 45 °C and *Enterococcus* and *Pediococcus* at 50 °C.
- The production of arginine dihydrolase on the M17 PCB medium **[26]** and the hydrolysis of esculin according to the method of **[27]**.
- The ability to grow on M17 and MRS medium in the presence of NaCl at different concentrations and different pH values was observed for 2 to 3 days of incubation. The isolates of the genera were tested as follows: *Streptococcus, Lactococcus,* and *Lactobacillus* at 2 % and 4 % NaCl and pH 4.5 and 6.5, *Leuconostoc* at 3 % and 6.5 % NaCl and pH 4.5, 4.8 and 6.5 and *Pediococcus* at 2 %, 3 %, 4 % and 6.5 % NaCl and pH 4.2, 4.8, 7.0 and 8.0.
- Only the isolates of the *Lactococcus, Leuconostoc,* and *Pediococcus* genera were tested for the production of acetoin in skimmed milk medium according to the technique described by **[28]**.
- The production of dextran from sucrose on MSE solid medium **[18]** has been applied for *Leuconostoc* isolates only, and the use of citrate on medium **[29]** for *Streptococcus* and *Lactococcus* isolates.

III. RESULTS

1. Physicochemical analysis: The results of the physicochemical analysis have been presented in Table 2. Where the pH range for traditional cheeses (*Jben*) was from 4.24 ±0.07 to 4.54 ±0.16 with an average of 4.39 ±0.15, the titratable conventional cheese acidity samples varied from values as low as 66.29 ± 3.3 °D to values as high as 72.51 ± 3.02 °D, the average titratable acidity value was 69.4 ± 3.11 °D.

Table 2. pH and acidity of the samples of the traditional cheese (*Jben*).

	pH						**Acidity**					
	S1(B)	**S2(O)**	**S3(D)**	**S4(A)**	**S5(M)**	**M**	**S1(B)**	**S2(O)**	**S3(D)**	**S4(A)**	**S5(M)**	**M**
Mean	**4,39**	**4,54**	**4,54**	**4,24**	**4,24**	**4,39**	**69,4**	**72,51**	**72,51**	**66,29**	**66,29**	**69,4**
SD	**0,15**	**0,15**	**0,16**	**0,22**	**0,07**	**0,15**	**3,57**	**3,02**	**2,3**	**3,3**	**3,36**	**3,11**

S: Samples, SD: Standard Deviation, M: Mean.

2. Microbiological analysis:

2.1. Determination of microbiological quality: The average of the results obtained has a significant microbial load in terms of TAMF, coliforms, and yeasts, and the absence of molds, *Staphylococcus*, and *Salmonella*. The results of microbiological properties of traditional cheese (*Jben*) are presented in Table 3. The total number of the total aerobic mesophilic flora (TAMF) varied from 1.4 x 10^6 CFU/g and 14 x 10^6 CFU/g with an average of 6.34 x 10^6 CFU/g. The number of coliforms varied from 1.1 x 10^4 CFU/g to 2.3 x 10^4 CFU/g; the average number of coliform bacteria was 1.44 x 10^4 CFU/g. The total number of

yeasts varied from 1.2 x 10^4 CFU/g and 2.4 x 10^4 CFU/g with an average of 1.88 x 10^4 CFU/g. Molds and pathogenic bacteria *Staphylococcus* and *Salmonella* were not detected.

Table 3. Results of the microbiological analysis (CFU/g) of the samples from the *Jben*.

Analyses microbiologiques	S1(B)	S2(O)	S3(D)	S4(A)	S5(M)	M±SD	Norme
TAMF x 10^6	**2.2**	**1.4**	**2.10**	**12**	**14**	**6.34± 0.53**	**10^5/g**
Coliforms x 10^4	**1.2**	**1.4**	**1.1**	**1.2**	**2.3**	**1.44± 0.83**	**10/g**
Yeasts x 10^4	**2.3**	**2.4**	**1.2**	**1.4**	**2.1**	**1.88± 0.75**	**10^2/g**
Staphylococcus	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**0/1g**
***Salmonella* (1g)**	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**0/1g**
Molds	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**Abs**	**----**

S: Samples, M: Mean, SD: Standard Deviation, Abs: Absence.

2.2. Exploration of the biodiversity of the lactic microflora:

The mean values of the lactic flora on MRS and M17 medium are 2.28 x 10^6 CFU/g and 7.1 x 10^5 CFU/g, respectively, upon enumeration. We isolated 164 Gram-positive, and catalase-negative isolates (between 32 and 35 isolates per *Jben* sample) from our five *Jben* samples. The 164 isolates were arranged as follows, with dominance order: Among the lactobacilli (63 isolates, 38.4%), there are 51 isolates (31.1%) of mesophilic lactobacilli and 12 isolates (7.3%) of thermophilic lactobacilli; moreover, there are 43 isolates (26.4%) of lactic enterococci, 33 isolates (20.2%) of lactococci, 17 isolates (10.6%) of leuconostocs, and 8 isolates (4.4%) of pediococci. Tables 4 and 5 provide the morphological and physiological features of the putative genera of lactic acid bacteria.

Table 4. Morphological characters of the presumed genera of the isolated lactic acid bacteria.

Macromorphology	Micromorphology	Type of Fermentation	T(°C)	Groups (number of isolates)
White, round or lenticular colonies	**Coccis, diplococci and chain**	**Homofermentative**	**45**	**Lactic enterococci (43)**
White, round or lenticular colonies	**Coccis, diplococci and chain**	**Homofermentative**	**30**	**Lactococci (33)**
Transparent colonies are very small, round	**Coccis, oval, chain**	**Heterofermentative**	**30**	**Leuconostocs (17)**
Smooth rounded, grayish or whitish colonies	**Tetrad coccis**	**Homofermentative**	**30**	**Pediococci (8)**
Small white colonies with brown centers and domed	**Long coiled or filamentous sticks, isolated or in chains**	**Homofermentative**	**45**	**Thermophilic lactobacilli (12)**
Small white colonies, round or lenticular	**Small sticks in chains**	**Homofermentative + Heterofermentative**	**30**	**Mesophilic lactobacilli (51)**

Table 5. Physiological and biochemical profile of the isolated lactic acid strains.

Strain/ Character	*Enterococcus*	*Lactococcus*	*Leuconostoc*	*Pediococcus*	Mesophilic lactobacilli	Thermophilic lactobacilli
Gram	+	+	+	+	+	+
Form	Cocci	Cocci	Cocci	Cocci	Bacillus	Bacillus
Catalase	-	-	-	-	-	-
Growth at 30 °C	-	+	+	+	+	+
Growth at 45 °C	+	-	-	-	-	+
Growth at 6,5 % NaCl	+	+	+	+	+	+
Growth at PH =4,5	-	-	-	-	+	+
Growth at PH =6,5	+	+	+	+	+	+
Growth at PH =9,6	-	-	+	-	-	-
Distribution isolates	43 (26.4%)	33 (20.2%)	17 (10.6%)	8 (4.4%)	51 (31.1%)	12 (7,3%)

VI. DISCUSSION

1. Physicochemical analysis: The pH values are similar to those found by **[30]**. The acidity values are almost identical to those reported by **[31]** and **[32]**. A great variability observed for traditional Algerian cheeses and even based on limited data reflects the conventional dairy typicity transformations in each region, this requires a characterization of these traditional dairy products by respecting the specificity of each cheese including the *Jben* in the steppe regions.

2. Microbiological analysis:

2.1. Determination of microbiological quality: The results indicate a possibility of general contamination and a lack of hygiene, which requires corrective measures to reduce the microbial load and improve the safety and quality of the cheese. Many common enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7, and *Campylobacter* are transported in the intestinal tract of ruminants, including domestic animals used in dairy production, for example, cows, sheep, and goats. Effective cleaning procedures, including the removal of feces from the udders before milking and good manufacturing practices during the cheese-making process, can reduce the risk **[33]**, consumer requirements for traditional fermented milk products are generally increased due to their proven gastronomic quality and their positive effects on human health. However, the tightening of legislation on food safety is reflected in less flexibility of production, homogeneity of food production, and a loss of food diversity and traditional specificity. Therefore, the preparation of well-defined functional autochthonous sourdough cultures for the production of traditional cheeses under controlled conditions, using standardized traditional technology, is crucial **[34]**. According to the results obtained, in this research, the counts of TAMF, coliform bacteria, and Yeasts present in traditional cheese (*Jben*) were higher than the upper limits given by the European Commission (EC) **[35]**. It is not surprising to obtain high microbiological contents in cheese with artisanal manufacturing methods due to the use of unpasteurized milk **[36]**.

2.2. Exploration of the biodiversity of the lactic microflora: According to **[37]**, the lactic flora count on MRS and M17 medium yields average values that are comparable to those of conventional *Koopeh* cheese. Lactobacilli predominate, with lactic enterococci, lactococci, leuconostocs, and pediococci

following in order of importance, indicating a favorable microbial biodiversity for the production of high-quality *Jben*. Similar average values were found when lactic flora on MRS and M17 media was counted in traditional *Koopeh* cheese samples. This suggests that a rich microbial diversity is beneficial for the production of high-quality *Jben* **[37]**. These results highlight the importance of microbial biodiversity, including lactobacilli, lactic enterococci, lactococci, leuconostocs, and pediococci, in assuring the quality and distinctive qualities of *Jben* cheese, and highlight the role of particular bacterial strains in the fermentation and maturation processes.

V. CONCLUSION

With a focus on food safety and the optimization of fermentation conditions to improve the sensory quality of the product, the predominant presence of lactobacilli, followed by lactic enterococci, lactococci, leuconostocs, and pediococci, indicates beneficial microbial biodiversity for the production of quality *Jben*. Many cutting-edge approaches may be applied to gain a more thorough investigation of isolates at the species and subspecies stage as well as to assess their technical and health interests:

• Polymerase Chain Reaction (PCR) and the Analytical Profile Index (API) gallery.
• Research on technical skills and probiotic characterization.

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