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Characterisation And Antibacterial Screening Of Indigenous Lactic Acid Strains Against Food Pathogens

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Abstract:
In this study, a total of 70 isolates were obtained from raw goat's and cow's milk cultured on MRS and M17 media, 27 of which exhibited the characteristics of lactic acid bacteria. Identification by MALDI-TOF MS revealed that these isolates mainly belonged to the species Enterococcus faecium (55.55%), Pediococcus pentosaceus (22.22%), Lactococcus lactis (7.40%) and Lactobacillus acidophilus (3.70%), while three isolates (11.11%) could not be reliably identified.
Evaluation of antibacterial activity against Escherichia coli ATCC 25922 led to the selection of 11 lactic acid strains. However, tests carried out on Staphylococcus aureus ATCC 25923 reduced this number to five strains, which were selected for further investigation. This work will focus on characterising the antimicrobial metabolites produced, particularly bacteriocins, and evaluating their in situ efficacy in food matrices with a view to potential applications in biopreservation.
Keywords: *Raw milk; Lactic acid bacteria; Antibacterial activity; MALDI-TOF; Indigenous.*

1.INTRODUCTION

Lactic acid bacteria are a heterogeneous group of species that share one major characteristic: the production of lactic acid as their main fermentation product. They are distinguished by their Gram-positive nature, their lack of catalase activity and their inability to form spores (Vos et al., 2011). In addition to lactic acid, these microorganisms synthesise a variety of secondary metabolites that contribute, directly or indirectly, to functions of clinical and technological interest (Wang et al., 2021). For these reasons, lactic acid bacteria are widely used in the biomedical and agri-food sectors. Food additives play a crucial role on an industrial scale, particularly in the agri-food industry, where they are used mainly for preservation purposes, but also to improve organoleptic properties (Singh and Kumar, 2023). However, the vast majority of these additives are chemical in origin and produced synthetically. In a context where consumer demand for more natural, healthy and chemical-free foods is constantly growing, the search for biological alternatives is becoming essential (Timothy et al., 2021; Yang et al., 2023).

In Algeria, the availability of vast unurbanised areas favours animal husbandry, particularly species known for their meat and milk production. While camel, cattle, sheep and goat farming are widespread, the cattle and goat sectors remain the most productive in terms of milk, particularly in the northern and high plateau regions (MEDJAHED et al., 2024). The value of these resources lies mainly in their processing, but their microbiological potential remains under-exploited. It is with this in mind that our work aims to promote local microbiological resources as a sustainable and safe

alternative to chemical preservatives, while helping to reduce their overconsumption and improve the socio-economic balance linked to imports.

This study has the following objectives:

• To isolate, purify and identify indigenous lactic acid bacteria present in raw goat's and cow's milk.

• To evaluate the antagonistic activity of selected lactic acid strains against two foodborne pathogenic bacteria, one Gram-negative and the other Gram-positive.

2.MATERIALS AND METHODS

2.1.Isolation of lactic acid bacteria

Lactic acid bacteria were isolated from raw goat's and cow's milk from different locations in the wilaya of Blida. The milk samples were first incubated at 37°C and 44°C to promote the enrichment of mesophilic and thermophilic lactic flora. Successive decimal dilutions were then prepared up to 10^{-9}. A volume of 1 mL taken from the last five dilutions (10^{-5} to 10^{-9}) was seeded on MRS and M17 media. The plates were incubated at the same temperatures used for enrichment (37°C or 44°C). Each well-isolated and distinct colony was successively subcultured at least three times until a pure culture was obtained. Purity was verified by Gram staining. Isolates with lactic acid bacteria characteristics were preserved in selective broths with 30% glycerol and stored at -20°C (Samelis et al., 1994).

2.2. Preliminary biochemical tests for the identification of lactic acid isolates

2.2.1. Catalase Test

One colony from each pure culture was mixed with one drop of hydrogen peroxide (H_2O_2 1/10 v/v). The appearance of gas evolution was interpreted as a positive reaction, indicating the presence of the catalase enzyme (Djelloul Daouadji, 2021).

2.2.2. Oxidase Test

One colony from each isolate was spread on an oxidase disc. The appearance of a dark purple color was considered a positive reaction, revealing the presence of the oxidase enzyme.

2.3.Proteomic identification of presumptive lactic acid isolates

Presumptive lactic acid bacteria isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a proteomic approach targeting cell wall–associated proteins. The Extended extraction protocol was employed. For each isolate, a colony obtained from a pure culture was applied onto a designated spot of the target plate, followed by the sequential addition of formic acid and the HCCA matrix, which were allowed to air-dry. After complete drying and stabilization of the preparation, the target plate was introduced into the spectrometer for MALDI-TOF MS analysis (Seng et al,2009).

2.4. Antibacterial screening of lactic acid strains

The antibacterial activity of the lactic acid strains was assessed against two pathogenic bacteria (one Gram-positive and one Gram-negative) using the direct double-layer spot method as described by Hammadecheet al. (2025). For each lactic strain, a cell suspension was prepared at a concentration equivalent to 10^8 CFU/mL ($OD_{600} \approx 0.1$). A 5-µL aliquot of each suspension was spotted onto MRS or M17 agar plates and incubated at either 37 °C or 44 °C for 24 h.

The indicator strains Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were adjusted to a final concentration of 10^6 CFU/mL in soft TSA agar. This mixture was then poured as an upper layer over the previously incubated spots. Following 24 h of incubation at 37 °C, the inhibition zones surrounding the spots were measured using a digital caliper.

The antibacterial activity was evaluated using the double-layer spot method (HAMMADECHE et al., 2025). Lactic strains (10^8 CFU/mL) were spotted (5 µL) on MRS/M17 agar and incubated. Indicator strains (E. coli ATCC 25922, S. aureus ATCC 25923) adjusted to 10^6 CFU/mL in soft TSA were poured over the spots. After 24 h incubation at 37°C, inhibition zones were measured.

2.5.Statistical analysis

The experiments were performed in duplicate, and the results are expressed as mean ± standard deviation.

3.RESULTS AND DISCUSSION

3.1 Isolation and selection of lactic acid bacteriaI:

A total of 70 isolates were obtained from MRS and M17 media, the distribution of which is shown in Table 1. Based on the morphological and biochemical characteristics studied, 27 isolates (38.57%) were presumed to belong to lactic acid bacteria (Gram-positive, catalase- and oxidase-negative, non-spore-forming) in accordance with the criteria described by Nawaz and Jagadeesh (2019).

Morphologically, different shapes were observed: bacilli, coccobacilli and cocci. Their distribution shows that 11.11% (3 isolates) were bacilli arranged in short chains, 51.85% (14 isolates) were coccobacilli most often forming short chains or diploids, and 37.04% (10 isolates) were cocci associated in short chains or diploids.

Raw milk is a rich polymicrobial ecosystem, harbouring a wide variety of microorganisms. The diversity observed in this study is consistent with previous work by Hammadeche et al. (2025), Bouchibane et al. (2022) and Madi and Boushaba (2017).

This matrix is distinguished by its richness in nutrients, particularly carbohydrates, which makes it a favourable environment for the development of indigenous microflora, especially the bacteria responsible for lactic fermentation. The latter mainly produce lactic acid as the final metabolite (Bluma and Ciprovica, 2015), thus contributing to the transformation of milk during fermentation. In addition to lactic acid, other compounds such as acetic acid, hydrogen peroxide (H_2O_2), carbon dioxide (CO_2) and various bacteriocins are also synthesised, enhancing the biopreservation properties of fermented milk (Wang et al., 2021; Muhialdin et al., 2011).

Table 1: Distribution of isolates obtained according to morphological and biochemical characteristics.

Number of isolates	Macroscopicappearance			Aspect microscopique			Tests biochimique	
	Color	Shape	Size	Gram	Shape	Arrangement	Catalase	Oxydase
30	Whitish	Lenticular	>5mm	+	Bacille	Chainette	+	+/-
13	Whitish	Lenticular	1 – 3 mm	+	Cocci	Amas	+	+
03	Whitish	Lenticular	1 – 3 mm	+	Bacille	Chainette + diplobacilla	-	-
14	Whitish	Lenticular	1 – 3 mm	+	Coccobacill e	Short chain + diploid	-	-
10	Whitish	Lenticular	1 – 3 mm	+	Cocci	Chainette + diplococcus	-	-

3.2. Proteomic identification of pre-selected lactic acid isolates:

The 27 preselected isolates belonging to lactic acid bacteria were identified using MALDI-TOF MS. The detected species were distributed as follows: Enterococcus faecium accounted for 55.55% of the isolates (15 strains), Pediococcuspentosaceus for 22.22% (6 strains), Lactococcus lactis for 7.40% (2 strains), and Lactobacillus acidophilus for 3.70% (1 strain), while three isolates could not be reliably identified (Table 02). The score values ranged from 1.295 to 2.348; among them, 24 strains (88.88%) had a score ≥ 1.729 and 11 strains a score ≥ 2.014.

Table 2 : Identification of pre-selectedlacticacidstrains.

Isolat	Source	T° d'isolement	Espèce identifiée	Valeur score
LV1M2S1	Cow	37°C	Lactococcus lactisssplactis	2,189
LC2T1S5	Goat	44°C	Enterococcus faecium	2,057
LC2T2S6	Goat	44°C	Enterococcus faecium	2,119
LC2T2S10	Goat	44°C	Enterococcus faecium	2,48
LC1T1S2	Goat	44°C	Lactobacillus acidophilus	2,07

LC2T1S9	Goat	44°C	Enterococcus faecium	2,348
LV1T2S1	Cow	44°C	Enterococcus faecium	2,277
LC1M1S2	Goat	37°C	Pediococcuspentosaceus	2,231
LC1M1S4	Goat	37°C	Enterococcus faecium	2,014
LC1M1S8	Goat	37°C	Pediococcuspentosaceus	2,118
LC1M1S7	Goat	37°C	Pediococcuspentosaceus	2,104
LC1M2S5	Goat	37°C	Enterococcus faecium	1,923
LC1M1S6	Goat	37°C	Enterococcus faecium	1,731
LV1M2S3	Cow	37°C	Lactococcus lactis	1.969
LC2T1S8	Goat	44°C	Enterococcus faecium	1,931
LC2T1S2	Goat	44°C	Enterococcus faecium	1,75
LC1M1S1	Goat	37°C	Enterococcus faecium	1,927
LC1M2S4	Goat	37°C	Enterococcus faecium	1,729
LV1M2S2	Cow	37°C	Lactococcus lactis	1,807
LC1M1S5	Goat	37°C	Pediococcuspentosaceus	1,802
LC1M1S3	Goat	37°C	Pediococcuspentosaceus	1,903
LC2T1S4	Goat	44°C	Enterococcus faecium	1,738
LC1T1S3	Goat	44°C	Enterococcus faecium	1,769
LC1T1S1	Goat	44°C	Pediococcuspentosaceus	1,769
LV1M1S10	Cow	37°C	Identification non fiable	1,337
LV1T2S2	Cow	44°C	Identification non fiable	1,295
LC2T1S7	Goat	44°C	Identification non fiable	0

The obtained collection was diverse and covered most groups of lactic acid bacteria, with a clear predominance of E. faecium (Figure 1). Such diversity has already been reported in previous studies, particularly those of Badis et al. (2004) and Madi and Boushaba (2017). Moreover, our observations are consistent with those of **Boudjelthia et al. (2023)**, who also reported a strong predominance of the genera Enterococcus, Lactococcus, and Pediococcus in Algerian raw milk.

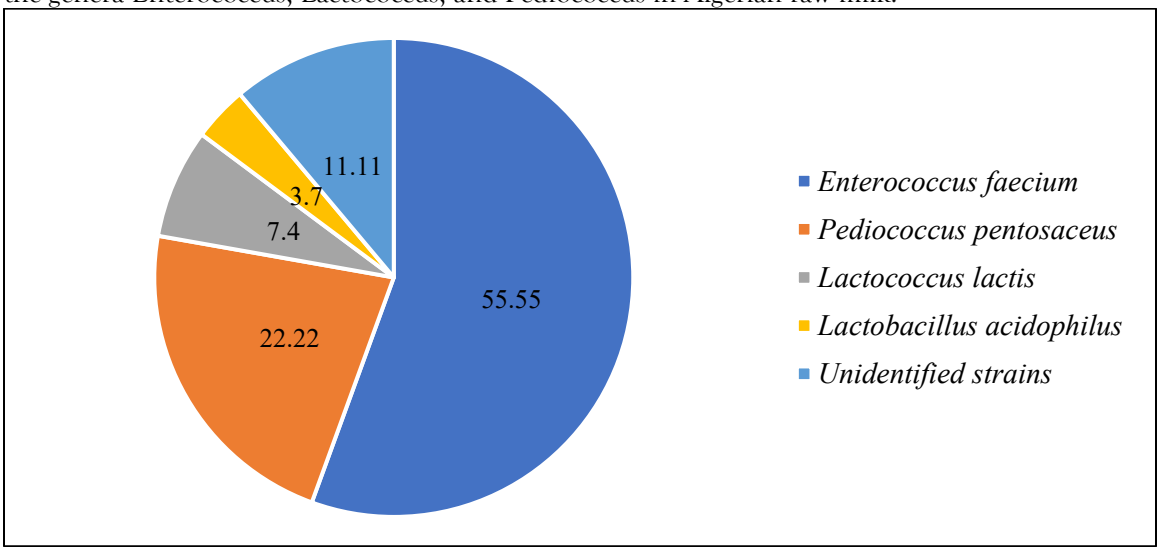

Figure 1 : répartition (%) des espèces de bactéries lactiques isolés de lait cru de vache et de chèvre.

Our findings also show notable similarities with those of **Kocsis et al. (2025)**, who investigated the microbial diversity of dairy products using MALDI-TOF MS. In their study, the authors reported a strong representation of species belonging to the genera Enterococcus, Lactococcus, and Pediococcus, confirming that these groups frequently dominate dairy environments, whether in raw milk or fermented products. They also highlighted that these genera often display considerable intra-

species variability while remaining reliably identifiable by MALDI-TOF due to their characteristic protein profiles. The similarity between our results and those of Kocsis et al. suggests that the structure of the lactic microbiota observed in our study follows microbial patterns commonly described in dairy matrices.

According to Liu et al. (2014) and Klaenhammer et al. (2002), the lactic microflora of milk belongs mainly to the phylum Firmicutes, which includes the genera Lactobacillus, Pediococcus, Lactococcus, Enterococcus, and Streptococcus. The results obtained in the present study are therefore in full agreement with the available literature.

3.3. Antibacterial activity

In a first step, the preselected lactic acid strains were evaluated against Escherichia coli ATCC 25922. Variable inhibition zone diameters ranging from 9.5 ± 0.5 mm to 33 ± 8 mm (spot diameter ≈ 6 mm included) were recorded for 40.74% of the strains. Conversely, 59.26% of the strains exhibited no inhibitory activity, resulting in a complete absence of inhibition zones (Table 03 and Figure 02).

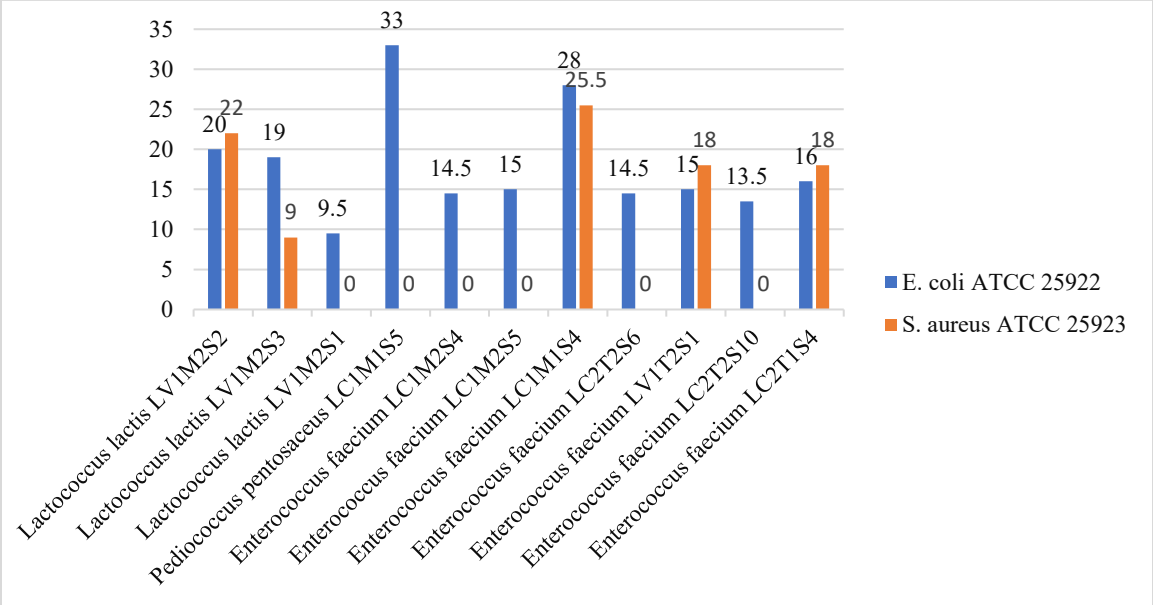

Figure 2: Antibacterial activity of lactic acid strains against E. coli ATCC 25922 and S. aureus ATCC 25923.

In a second step, the lactic acid strains that showed antagonistic activity against E. coli ATCC 25922 were subsequently evaluated against Staphylococcus aureus ATCC 25923. Among the eleven strains tested, five displayed inhibitory activity, with inhibition zone diameters ranging between 13 ± 0 mm and 25.5 ± 5.5 mm, as shown in Table 03 and Figure 02.

Table 03: Mean inhibition zones formed against E. coli ATCC 25922 and S. aureus ATCC 25923.

Lacticacidstrain	Average inhibition zones (mm)	
	E. coli ATCC 25922	S. aureus ATCC 25923
Lactococcus lactis LV1M2S2	20 ± 0	22 ± 3
Lactococcus lactis LV1M2S3	19 ± 1	9 ± 0
Lactococcus lactis LV1M2S1	9.5 ± 0.5	-
Pediococcuspentosaceus LC1M1S5	33 ± 8	-
Enterococcus faecium LC1M2S4	14.5 ± 0.5	-
Enterococcus faecium LC1M2S5	15 ± 1	-
Enterococcus faecium LC1M1S4	28 ± 8	25.5 ± 5.5

Enterococcus faecium LC2T2S6	14.5 ± 2.5	-
Enterococcus faecium LV1T2S1	15 ± 3	18 ± 0
Enterococcus faecium LC2T2S10	13.5 ± 0.5	-
Enterococcus faecium LC2T1S4	16 ± 0	18 ± 0

(-) : negativeantibacterialactivity.

The strains demonstrating a dual spectrum of activity (against both Gram-negative and Gram-positive bacteria) belonged to the species Lactococcus lactis and Enterococcus faecium. The antibacterial potential of these species is widely documented in the literature. However, certain lactic acid bacteria, particularly some Enterococcus strains, have lost their GRAS status due to the emergence of antimicrobial resistance—a phenomenon linked to agroecological imbalances (notably the overuse of pesticides) and the excessive use of antibiotics in veterinary and human medicine. Therefore, a thorough assessment of their safety profile remains essential.

This study represents a preliminary step aimed at selecting indigenous lactic acid bacteria strains with targeted activity against foodborne pathogenic bacteria, with a view to their potential application in biopreservation. Such an approach could contribute to reducing the use of chemically synthesized industrial preservatives.

In the continuation of this work, the next step will involve characterizing the active agent responsible for the observed antibacterial activity, particularly through the purification and identification of bacteriocins, followed by evaluating their in situ efficacy to confirm their potential application.

4. CONCLUSION

This study is part of an effort to valorize local microbiological resources through the isolation of indigenous lactic acid bacteria from raw goat's and cow's milk collected from different localities within the Blida region. A collection of 27 strains exhibiting the typical characteristics of lactic acid bacteria was preselected and subsequently identified by MALDI-TOF MS as belonging mainly to the species Enterococcus faecium, Lactococcus lactis, Pediococcuspentosaceus, and Lactobacillus acidophilus. These findings highlight the diversity of the lactic microbiota present in the raw milk of both animal species.

Eleven strains were then selected based on their initial antagonistic activity against a Gram-negative pathogenic bacterium (Escherichia coli ATCC 25922). A second screening aimed at identifying strains with a dual spectrum of activity was carried out against a Gram-positive bacterium (Staphylococcus aureus ATCC 25923). Following this selection process, five strains belonging to Enterococcus faecium and Lactococcus lactis were retained due to their strong antibacterial potential. These strains represent promising candidates for future applications in biopreservation, with the objective of limiting food spoilage and reducing the impact of foodborne pathogens through control by safe lactic acid bacteria.

Perspectives for furtherdevelopment:

- Purification and characterization of active antimicrobial metabolites, particularly bacteriocins.
- In-depth genomic identification of selected strains through whole-genome sequencing (NGS) and bioinformatic exploration of genes of technological and functional interest.
- Determination of the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of purified bacteriocins, followed by in situ efficacy assays in different food matrices.
- Lyophilization, standardization, and formulation of preparations with technological or biopreservative interest.

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23. The obtained collection was diverse and covered most groups of lactic acid bacteria, with a clear predominance of E. faecium (Figure 1). Such diversity has already been reported in previous studies, particularly those of Badis et al. (2004) and Madi and Boushaba (2017). Moreover, our observations are consistent with those of Boudjelthia et al. (2023), who also reported a strong predominance of the genera Enterococcus, Lactococcus, and Pediococcus in Algerian raw milk.
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26. Proteomic identification

27. MALDI-TOF identified isolates as Enterococcus faecium (55.55%), Pediococcus pentosaceus (22.22%), Lactococcus lactis (7.40%), Lactobacillus acidophilus (3.70%), with 11.11% unidentified. Most isolates scored ≥ 1.729. The diversity is consistent with Badis et al. (2004) and Madi & Boushaba (2017). Lactic microflora mainly belong to Firmicutes, including Lactobacillus, Pediococcus, Lactococcus, Enterococcus, and Streptococcus (Liu et al., 2014; Klaenhammer et al., 2002).

28. Antibacterial activity

29. Against E. coli ATCC 25922, 40.74% of strains showed inhibition (9.5–33 mm). Against S. aureus ATCC 25923, five strains maintained activity (13–25.5 mm). Dual-activity strains belonged to Lactococcus lactis and Enterococcus faecium. Enterococci require safety evaluation due to resistance concerns.

30. This work forms the basis for selecting indigenous lactic strains with biopreservation potential.

31. Conclusion

32. This study isolated and characterised lactic acid bacteria from raw goat and cow milk in Blida. Twenty-seven strains were identified, mainly Enterococcus faecium, Pediococcus pentosaceus, Lactococcus lactis, and Lactobacillus acidophilus. Eleven strains showed activity against E. coli, and five against S. aureus. These strains represent promising candidates for biopreservation.

33. Further research:

- Purification and characterisation of bacteriocins
- Whole-genome sequencing
- Determination of MIC/MBC
- Lyophilisation and formulation of biopreservative preparations