Bulgarian Journal of Agricultural Science, 32 (No 4) 2026, 948–965

Preliminary study on the antagonistic effect of lactic acid bacteria isolated from dried and salted camel meat (Kadid) – A traditional processed meat product from Southwestern Algeria – against selected microbial contaminants

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Abstract

Benyagoub, E., Bounegabi, K., Kerkoub, M., Bessadet, C., Touati, M. & Chachoua, F. Z. (2026). Preliminary study on the antagonistic effect of lactic acid bacteria isolated from dried and salted camel meat (Kadid) – A traditional processed meat product from Southwestern Algeria – against selected microbial contaminants. *Bulg. J. Agric. Sci., 32*(4), 948–965

Camel meat, as a food source, contributes significantly to achieving food security in the southern regions of Algeria. It is used to prepare a traditionally processed meat, known locally as 'Kadid', which involves drying fresh camel meat in the open air to extend its shelf life. This preservation ability is attributed to several factors, including not only physicochemical parameters, but also microbial factors. This study aims to evaluate the antagonistic effect of lactic acid bacteria (LAB) strains, isolated from Kadid, against selected microbial contaminants.

LAB strains and microbial contaminants were isolated using the spread plate technique on appropriate agar media for each microbial group. After purification, microbial contaminants were identified through a series of biochemical tests and then subjected to susceptibility testing on Mueller-Hinton agar using the Kirby-Bauer disc diffusion method. The *in vitro* evaluation of the antibacterial effect of the LAB isolates was conducted by spot testing, while the antifungal effect was assessed using the mycelial radial growth method on agar and the biomass method in a broth medium.

A total of 13 microbial contaminants and 9 LAB strains were isolated. Bacterial contaminants showed variable antibiotic susceptibility between strains. Several antibiotics tested have retained their antibacterial action. Most of the isolated LAB strains showed positive proteolytic activity (5/9, i.e., 55.5%), and good acidifying power, with lactic acid production ranging from 1.86 to 3.24g/L. This acidifying ability is likely the source of the moderate to strong antibacterial effect of the LAB strains. Additionally, the LAB strains exhibited an antifungal effect, with inhibition rates of radial growth ranging from 5% to 25%.

Dried and salted camel meat can serve as a source of LAB strains with valuable technological properties, making them suitable for use in fermentation industries and other applications.

Keywords: antagonistic effect; Kadid; lactic acid bacteria; microbial contaminants; Southern Algeria

Introduction

In arid regions with harsh climates, camel meat is an excellent source of high-quality protein, containing all essential amino acids in quantities similar to those found in lamb meat (Hoffman, 2008; Benyagoub and Mammeri, 2023). This applies not only to its major biochemical components, but also to its mineral content, which is comparable to other red meats, though certain elements may differ. Brahimi et al. (2020) and Djenane (2023) further noted that camel meat is more affordable than other red meats, making it accessible to disadvantaged population groups and providing them with essential animal proteins.

Drying is one of the traditional methods of preserving red meat, adopted in the past due to the lack of modern preservation techniques such as refrigeration and freezing. This process involves transforming fresh meat into dried meat, commonly known as 'Kadid or El Guedid', a traditional product that is salted, seasoned, and dried at room temperature, away from direct sunlight. During the maturation process, the physicochemical and microbiological parameters of the dried meat undergo significant changes (Benyagoub and Bessadet, 2023; Benyagoub and Mammeri, 2023).

A significant portion of microorganisms, including bacteria and fungi from the saprophyte group, contaminate carcasses during various stages of slaughter and can cause meat spoilage. Furthermore, the presence of pathogenic bacteria responsible for foodborne illnesses is often due to hygiene deficiencies (Benyagoub et al., 2017; Benyagoub et al., 2018a).

However, within this microbial diversity that develops during the maturation of dried meat, there are microorganisms of technological interest, namely lactic acid bacteria, for which the meat provides a favorable environment for proliferation (Benlacheheb et al., 2019). Interactions can occur between these bacteria and other microorganisms present in the meat.

Lactic acid bacteria (LAB) are a group of beneficial bacteria that share similar properties and produce lactic acid as the primary end product of fermentation (Benyagoub, 2023b; Razni et al., 2024). Various technologies utilize LAB strains to meet the needs of the food industry and beyond. There is a growing trend to reduce the use of chemicals and heat treatments in food preservation, while maintaining or even enhancing sensory properties (Rodgers, 2001; Vermeiren et al., 2004).

Among the advantages of this technique are the enhancement of organoleptic characteristics in fermented products, the extension of their shelf life, and the reduction of meat wastage (Falowo, 2023). Preservation is achieved through the synthesis of various metabolites with antibacterial properties (Dortu and Thonart, 2009).

To better understand the interaction between LAB strains and microbial contaminants, this study aims to evaluate, *in vitro,* the antimicrobial effects of LAB strains isolated from dried and salted camel meat against selected microbial contaminants.

Materials and Methods

Sampling

Dried and salted camel meat, locally known as Kadid or El Guadid, is one of the ancient food products prepared using a traditional preservation method that dates back several decades, if not centuries. In Southwest Algeria, Kadid is made by dehydrating meat from the one-humped camel (*Camelus dromedarius*), which is then used in many traditional dishes (Bouchefra et al., 2022; Benyagoub and Bessadet, 2023; Benyagoub and Mammeri, 2023; Benyagoub et al., 2024).

To prepare Kadid, fresh camel meat is cut, salted, seasoned with a few spices (such as cumin, pepper), and then dried at room temperature in the shade (Benyagoub and Bessadet, 2023). The Kadid samples analyzed in this study were obtained from families in the Bechar region (Figure 1).



Fig. 1. Dried and salted camel meat sample (Kadid) (Original, 2024) (Photo: E. Benyagoub)
Source: Authors' own elaboration

Isolation of microbial contaminants

Microbial isolation was carried out in accordance with the standards established by the American Public Health Association (Salfinger and Tortorello, 2015). A stock solution of dried and salted camel meat was prepared, and a series of dilutions were made using buffered peptone water (ISO 8261, 2001), to isolate the following microbial param-

eters, excluding *Pseudomonas* spp, which was isolated from chilled camel meat:

- *Enterobacteriaceae* strains, Staphylococci, and fungal flora.
- Lactic acid bacteria strains.

The analytical approach for microbial isolation was as follows:

- ***Enterobacteriaceae*** strains were isolated on Mac Conkey agar and Hektoen Enteric agar media at 30 and 44°C for 24 to 48 hours (JORA n.72, 2017).
- **Staphylococci** strains were isolated on Baird-Parker agar medium at 37°C for 24 hours (JORA n.68, 2014).
- ***Bacillus* spp** were isolated using Mossel agar medium at 37°C for 24 hours (Rachedi et al*.,* 2021; Benyagoub and Mammeri, 2023).
- **Fungal flora** was isolated using Oxytetracycline-glucose-yeast extract agar medium at 25°C for three to five days (JORA n.52, 2015).
- **Lactic acid bacteria (LAB)** strains were isolated after enrichment in M17 and MRS broths. M17 and MRS agars were then used to isolate mesophilic and thermophilic streptococci, as well as lactobacilli, at 30°C and 44°C, respectively. To inhibit fungal growth, 1% cycloheximide was added to the agar plates. The Petri dishes were incubated under microaerophilic conditions using an anaerobic gas jar pack system to lower the oxygen level (Benyagoub, 2022a; Benyagoub, 2022b; Benyagoub et al*.,* 2022a).
- ***Pseudomonas* spp** was isolated from chilled camel meat using cetrimide-nalidixic acid agar medium at 37°C for 24 hours (Benyagoub et al*.,* 2022a; Benyagoub, 2023a; Benyagoub and Mammeri, 2023).

To purify bacterial isolates, successive subculturing was performed using nutrient agar and nutrient broth for bacterial contaminants. M17 and MRS broth, as well as M17 and MRS agar slant tubes, were employed to purify streptococci and lactobacilli, respectively. Final confirmation of the isolates was based on their macroscopic and microscopic characteristics.

Identification of microbial isolates

Microbial contaminants

Pure bacterial isolates were identified through a serial phenotypic and biochemical tests, following the methods described by Tille (2018), Benyagoub et al*.* (2018b), Bendada et al. (2022), Benyagoub (2023a), and Benyagoub (2024a,b). The isolated molds were identified based on colony morphology, conidia, and conidiogenous cells using the microculture technique and the single-spore method, as described by Barnett and Hunter (1972), Pitt (1973), Ramirez (1982), Haris et al. (1989), Vermeiren et al. (2004), and Visagie et al. (2014).

Antibiotic susceptibility testing

The multidrug resistance (MDR) of bacterial contaminants was assessed using the Kirby-Bauer disc diffusion method, employing commercial antibiotic disks and Mueller-Hinton agar medium (Kirby et al*.,* 1956; Bauer et al*.,* 1959; Benyagoub et al*.,* 2020a; Benyagoub et al*.,* 2020b; Benyagoub et al*.,* 2022b; Benyagoub, 2024a).

After 18 to 24 hours of incubation at 37°C, the inhibition zones were measured and classified as susceptible (S), intermediate (I), or resistant (R) according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI, 2020).

In this study, the following antibiotics were tested against bacterial contaminants: Gentamicin (CN 10µg), penicillin (P 10µg), oxacillin (OX 1µg), cefoxitin (CX 30µg), chloramphenicol (C 30µg), sulfamethoxazole-trimethoprim (SXT 25µg), erythromycin (E 15µg), clindamycin (CD 2µg), ciprofloxacin (CIP 5µg), ampicillin (AMP 10µg), ofloxacin (OF 5µg), amoxicillin-clavulanic acid (AUG 30µg), amikacin (AK 30µg), amoxicillin (AML 2µg), tetracycline (TE 30µg), trimethoprim (TM 2,5µg), aztreonam (ATM 30µg), cefotaxime (CTX 30µg), ceftazidime (CAZ 10µg), piperacillin (PRL 100µg), ticarcillin-clavulanic acid (TTC 85µg), fosfomycin (FOS 50µg), nalidixic acid (NA 30µg), cefazolin (CZ 30µg), tobramycin (TOB 10µg), and imipenem (IMI 10µg).

Lactic acid bacteria (LAB)

Pure lactic acid bacteria (LAB) isolates were primarily identified through a series of tests, including the catalase test, microscopic examination in their native state using a bacterial suspension between a slide and coverslip, and Gram staining (Beveridge and Davies, 1983; Benyagoub, 2022b; Benyagoub, 2023b).

Technological potential of isolated LAB strains

The present study focused on two technological characteristics: acidifying power, measured by titratable acidity (g/L), and proteolytic activity, following the protocol described in the official methods of analysis by AOAC international (AOAC, 1990), Benyagoub (2022b), and Benyagoub (2023b).

Antagonistic effect of LAB strains

Antibacterial effect

The antagonistic effects of the isolated LAB strains were evaluated using the spot test on agar, as described by Fguiri

et al. (2017), Benyagoub (2022b), and Benyagoub (2023b). The antibacterial effect was assessed by measuring the diameter (in millimetres, mm) of the clear zones around the spots.

Antifungal effect

The antifungal effect of LAB strains was assessed using two methods: the plate assay and the broth culture assay, as described by Benyagoub (2022b), and Benyagoub (2023b).

- **Mycelial disc method (plate assay):** This was used as a qualitative method against a control. The antifungal effect (AE) was estimated by measuring the radial growth of the mycelial disc, expressed in millimeters (mm), according to the following equation:

$$AE(\%) = \left(\frac{Dt - D}{Dt}\right) \times 100,$$

where:

Dt is the average mycelial growth diameter of the control, and *D* is the average mycelial growth diameter of the test.

- **Fungal biomass method (broth culture assay):** This method was used as a quantitative assay by comparing the fungal biomass of treated samples with that of the control. After incubation, the fungal biomass was collected on filter paper and dried to constant weight. The fungal biomass (*FB*) was calculated as:

$$\mathrm{FB} = B_1 - B_0$$

where:

B_0 is the weight of the dry filter paper; and B_1 is the weight of the filter paper containing dried fungal biomass.

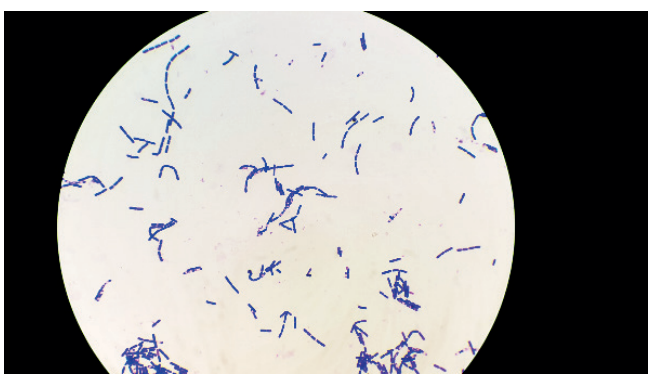
The antifungal activity (x) was expressed as percentage inhibition relative to the control using the following formula:

$$x = \left(\frac{FB_c - FB_t}{FB_c}\right) \times 100,$$

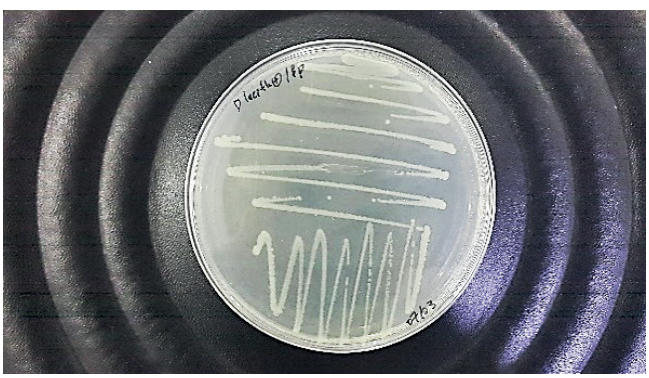
where:

FB_c is the fungal biomass of the control, and FB_t is the fungal biomass of the treated sample.

- **Conjugated effect:** Based on the results of the antifungal effects of the isolated LAB strains, those with the strongest antifungal properties were selected. The test was performed using the mycelial disc method, in which two perpendicular streaks of lac-



(a)



(b)



(c)

Fig. 2. Macro- and microscopic appearance of bacterial contaminants isolated from dried and salted camel meat (Original, 2024) (Photo: E. Benyagoub). (a): *Bacillus* sp; (b): *Enterobacter zakazakii*; (c): *Pseudomonas aeruginosa* (Chilled camel meat).

Source: Authors' own elaboration

tic acid bacteria were applied to form a framework surrounding the mycelial disc (binary combination). In the multiple combination, several streaks were made, each corresponding to a tested LAB strain (Razni et al., 2019).

All antagonistic tests were conducted in duplicate. For graphical presentations, OriginLab software (2018) was used to plot curves and histograms.

Results

Microbial contaminants

Identification of microbial isolates

Through microbial isolation using selective culture media followed by biochemical tests, the isolated microbial contaminants were identified as follows: *Klebsiella oxytoca, Enterobacter sakazakii, Enterobacter aerogenes, Enterobacter cloacae, Serratia odorifera, Escherichia coli, Pseudomonas aeruginosa, Bacillus* sp, *Staphylococcus hyaluronidase*, *Staphylococcus schleiferi,* and *Staphylococcus hyricus.* Additionally, two molds were isolated: *Penicillium expansum,* and *Aspergillus flavus* (Figures 2 and 3).

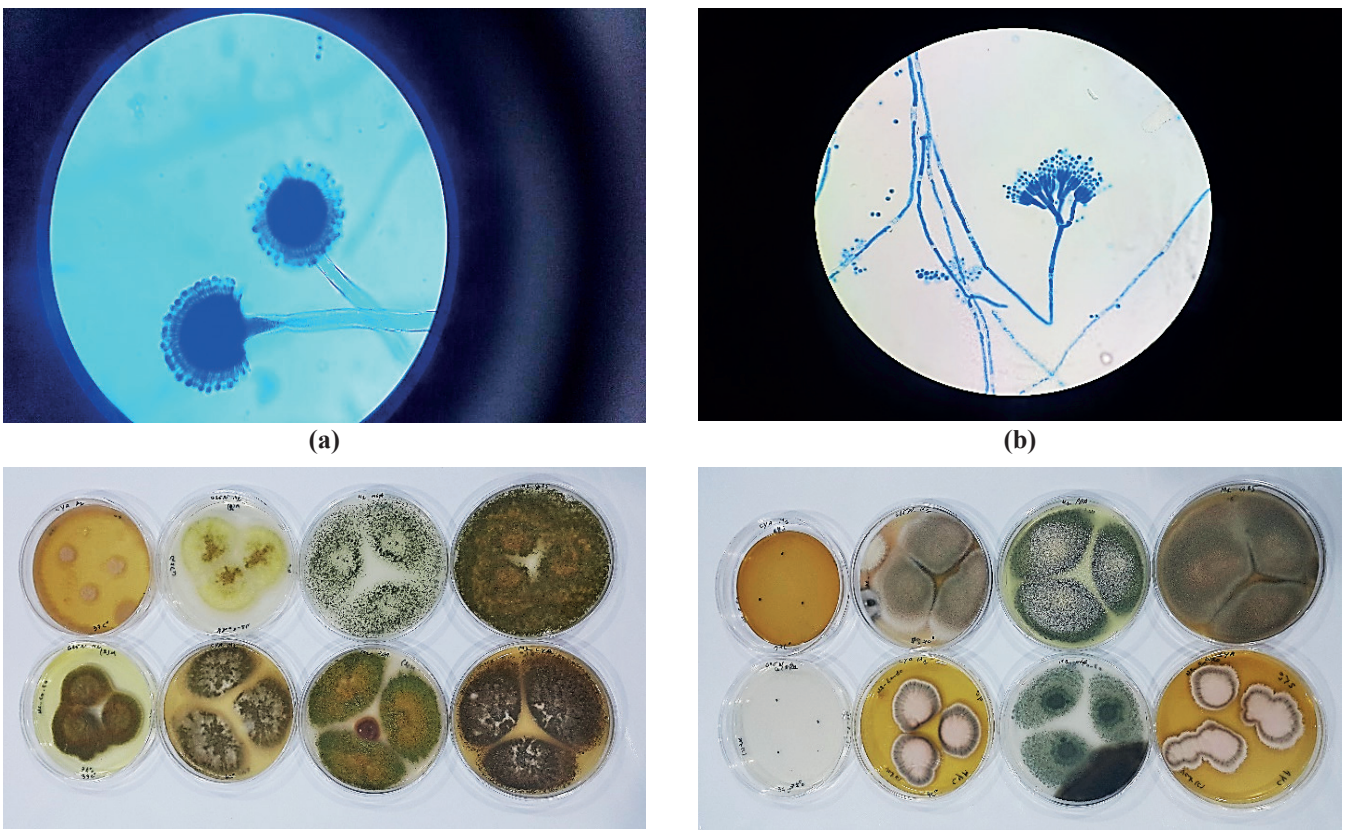
Antibiotic susceptibility test of bacterial contaminants

As per CLSI guidelines, the antibiotic resistance profiles of the tested bacterial contaminants are presented in Table 1.

The obtained results showed that most of the isolated *Enterobacteriaceae* strains were resistant to the following antibiotics: Ampicillin, amoxicillin+clavulanic acid, cefazolin, cefoxitin, and gentamicin. Meanwhile, staphylococci strains were resistant to penicillin, oxacillin, cefoxitin, and clindamycin (Figure 4). However, several antibiotics tested retained their antibacterial activity.

Lactic acid bacteria (LAB)

Isolation and phenotypic identification of LAB strains



(a) **(b)**

(c) **(d)**

Fig. 3. Microscopic appearance (microculture technique) and single spore method for mold identification (Original, 2024) (Photo: E. Benyagoub)

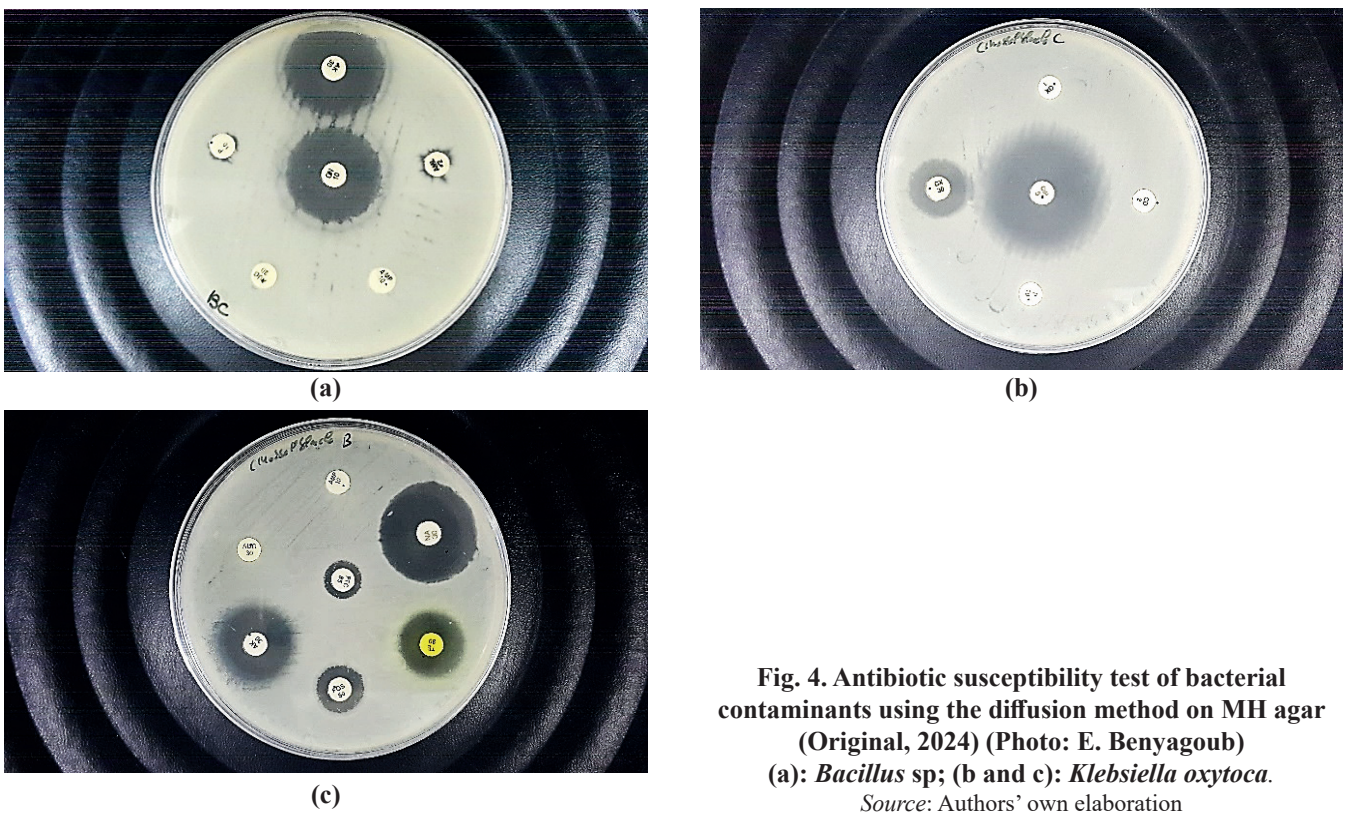
(a and c): *Aspergillus flavus*; (b and d): *Penicillium expansum*.

Source: Authors' own elaboration

Table 1. Multidrug-resistant profiles of bacterial contaminants isolated from dried and salted camel meat (Kadid) and *Pseudomonas aeruginosa* from chilled camel meat

Bacterial contaminants	Antibiotics
K. oxytoca	AMP-AUG-TTC-CZ-CN and susceptible to AK-TM-ATM-CX-CTX-CAZ-PRL-FOS-NA-CIP-TE-SXT-TOB-IMI-C
E. sakazakii	AMP-CX-CAZ-AUG-TTC-FOS-CZ-CN and susceptible to AK-TM-ATM-CTX-PRL-NA-CIP-OF-SXT-TOB
E. aerogenes	AMP-CX-CAZ-AUG-FOS-CZ-CN and susceptible to AK-TM-ATM-CTX-CAZ-NA-TE-OF-SXT-TOB-C
E. cloacae	AMP-CX-AUG-CZ-CN and susceptible to AK-TM-ATM-CTX-CAZ-PRL-TTC-FOS-NA-TE-OF-SXT-TOB-IMI-C
S. odorifera	AMP-CX-PRL-AUG-CZ-IMI and susceptible to AK-TM-ATM-CTX-CAZ-NA-TE-OF-SXT-TOB-C
E. coli	AMP-CX-ATM-CTX-CAZ-AUG-TTC-NA-CZ-TOB-CN and susceptible to TM-PRL-FOS-TE-OF-SXT-IMI-C
P. aeruginosa	CN-TTC and susceptible to AK-PRL-TOB-CAZ-IMI-OF-ATM-CIP
Bacillus sp	P-AML-AMP-AUG and susceptible to AK-CN
S. hyaluronidase	P-CX-OX and susceptible to SXT-TE-OF-CIP-E-CD-TM
S. schleiferi	P-CX-OX-TE-E-CD and susceptible to SXT-OF-CIP-TM
S. hyricus	P-CX-OX-CD and susceptible to SXT-TE-OF-CIP-E-TM

K. oxytoca*:** *Klebsiella oxytoca*; ***E. sakazakii*:** *Enterobacter sakazakii*; ***E. aerogenes*:** *Enterobacter aerogenes*; ***E. cloacae*:** *Enterobacter cloacae*; ***S. odorifera*:** *Serratia odorifera*; ***E. coli*:** *Escherichia coli*; ***P. aeruginosa: *Pseudomonas aeruginosa;* ***S. hyaluronidase*:** *Staphylococcus hyaluronidase;* ***S. schleiferi:*** *Staphylococcus schleiferi;* ***S. hyricus:*** *Staphylococcus hyricus.*
Source: Authors' own elaboration



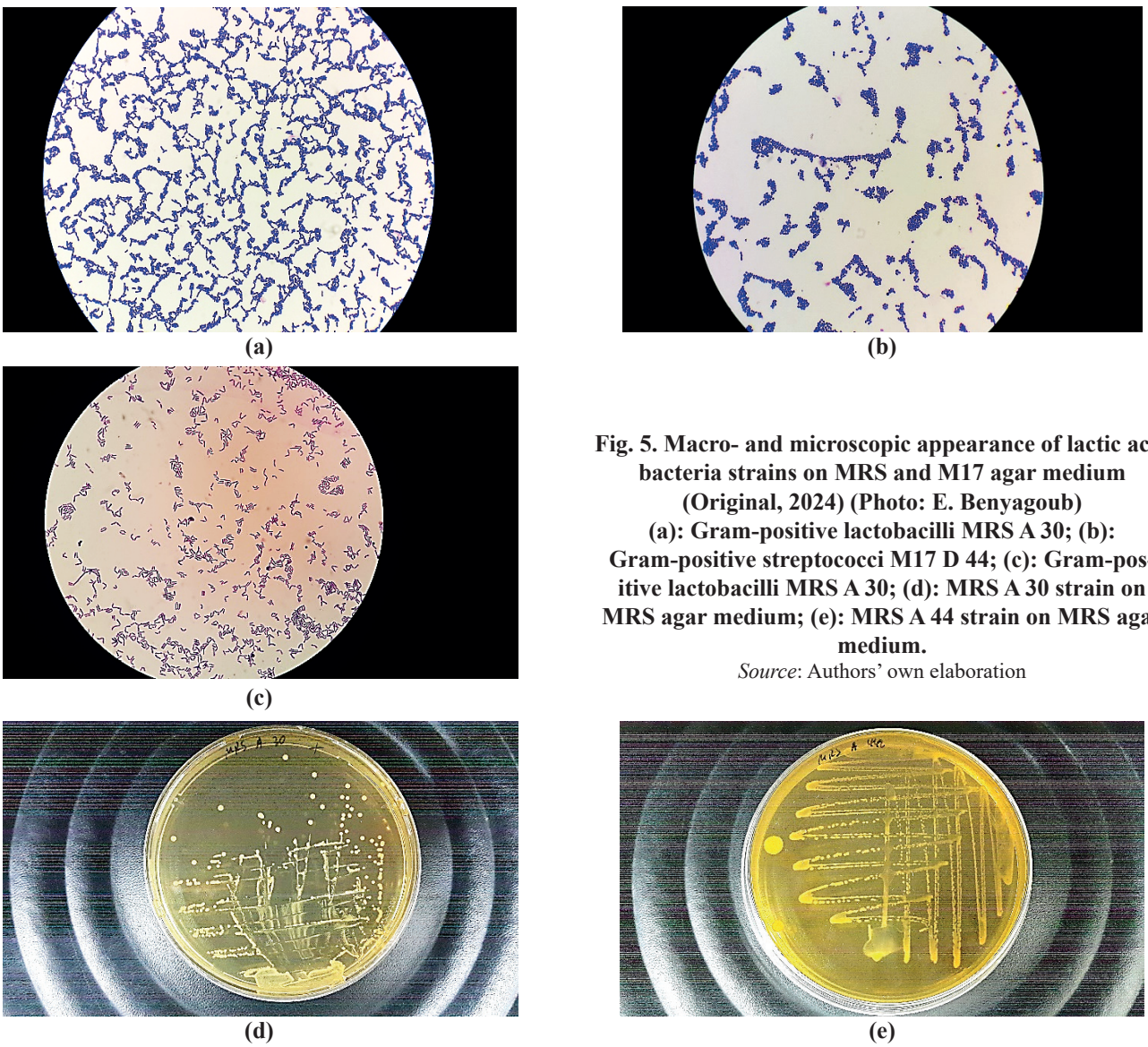
(a) **(b)** **(c)**

Fig. 4. Antibiotic susceptibility test of bacterial contaminants using the diffusion method on MH agar (Original, 2024) (Photo: E. Benyagoub)
(a): *Bacillus* sp; (b and c): *Klebsiella oxytoca*.
Source: Authors' own elaboration

Table 2. Microscopic and macroscopic appearance of LAB strains isolated from dried and salted camel meat (Kadid)

Characters / LAB strains	Temperature of isolation	Motility	Cell arrangement mode	Gram-type	Catalase test	Form	Macroscopic appearance
MRS A 30	30°C	Non-motile bacteria	single/pairs	Gram-positive bacteria	Negative test	Bacilli	Circular, whitish, raised, and smooth colonies
MRS A 44	44°C		chains			Cocci	
MRS C 30	30°C		chains			Cocci	
M17 C 44	44°C		chains			Cocci	
MRS D 30	30°C		single/pairs			Bacilli	
MRS D 44	44°C		chains			Cocci	
M17 D 44	44°C		chains			Cocci	
MRS F 30	30°C		single/pairs			Bacilli	
MRS TS 30	30°C		single/pairs			Bacilli	

Source: Authors' own elaboration



(a) **(b)** **(c)** **(d)** **(e)**

Fig. 5. Macro- and microscopic appearance of lactic acid bacteria strains on MRS and M17 agar medium (Original, 2024) (Photo: E. Benyagoub) (a): Gram-positive lactobacilli MRS A 30; (b): Gram-positive streptococci M17 D 44; (c): Gram-positive lactobacilli MRS A 30; (d): MRS A 30 strain on MRS agar medium; (e): MRS A 44 strain on MRS agar medium.

Source: Authors' own elaboration

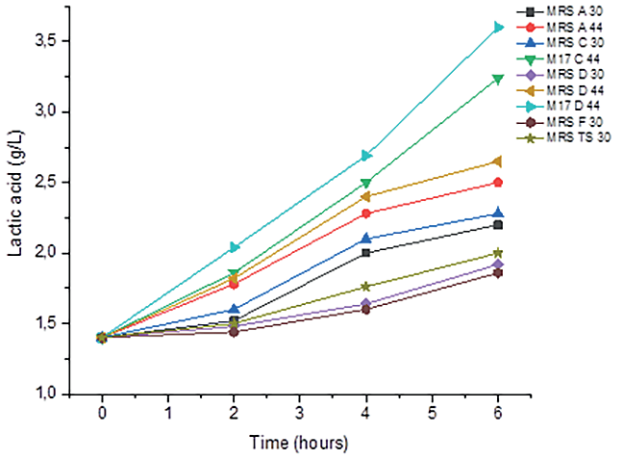


Fig. 6. Titratable acidity kinetics of skimmed milk as a function of time
Source: Authors' own elaboration

Through the phenotypic characteristics of LAB colonies (macro- and microscopic examination), as well as the catalase test result, nine lactic acid strains were isolated from dried and salted camel meat (Kadid) (Table 2). The colonies of the lactic isolates were whitish in color and smooth in texture (Figure 5).

Acidifying power

Figure 6 below shows the evolution of titratable acidity in skimmed milk during 6 hours of incubation with the tested LAB strains.

According to Figure 6 above, the acidifying power of the lactic acid bacteria was classified as medium to high and varied from one strain to another. The following LAB strains (M17 D44, M17 C44, MRS D 44, and MRS A 44) exhibited notable acidifying power, ranging from 2.5 to 3.5 g/L of lactic acid within 6 hours of incubation.

Proteolytic activity test

Proteolytic activity is an important technological characteristic of lactic acid bacteria, as it enables them to grow efficiently in protein-rich food matrices. The results of the proteolytic activity test are presented in Table 3.

The obtained results showed that out of the nine isolated LAB strains, five strains – M17 D44, M17 C44, MRS D 44, MRS A 44, and MRS A 30 – were protease producers, exhibiting proteolysis zone ranging from 10mm to 12mm (Figure 7).

Antagonistic effect of LAB strains against microbial contaminants

Antibacterial effect

The antibacterial effects of LAB strains against bacterial contaminants isolated from dried and salted camel meat (Kadid) are shown in Figures 8 and 9.

Table 3. Proteolytic activity results of LAB strains isolated from dried and salted camel meat (Kadid)

LAB / Proteolytic A.	MRS A 30	MRS A 44	MRS C 30	M17 C 44	MRS D 30	MRS D 44	M17 D 44	MRS F 30	MRS TS 30
Proteolysis zone (mm)	10 (+)	12 (+)	(-)	12 (+)	(-)	10 (+)	12 (+)	(-)	(-)

Proteolytic A.: Proteolytic activity; **LAB:** Lactic acid bacteria; **(-):** Negative reaction (Proteolysis zone <5mm); **(+):** Positive reaction (Proteolysis zone ≥5mm).
Source: Authors' own elaboration

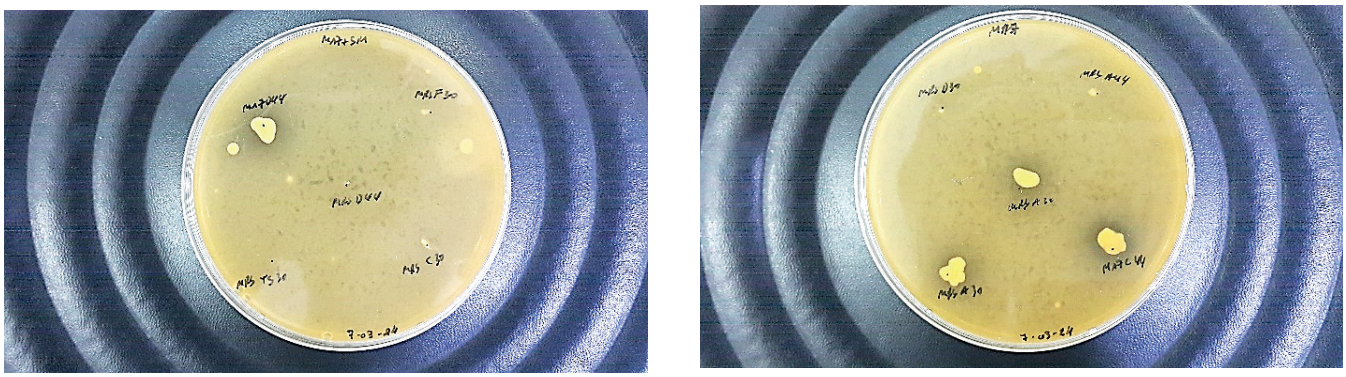
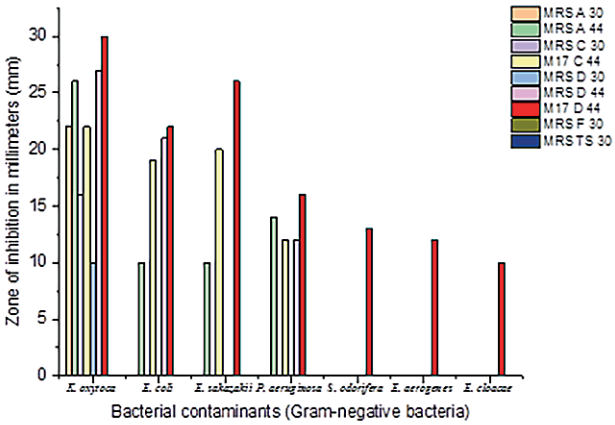
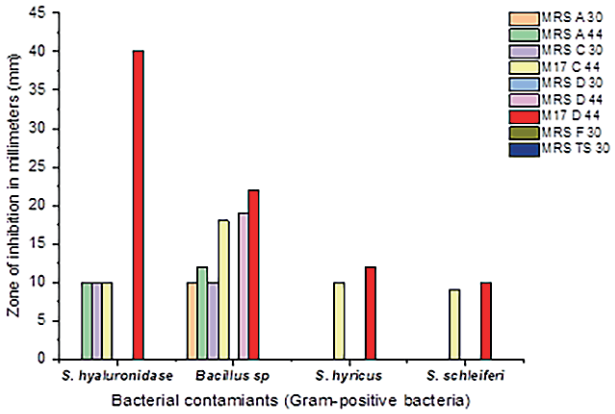


Fig. 7. Proteolytic activity of isolated LAB strains on milk-enriched agar medium (Original, 2024) (Photo: E. Benyagoub)
Source: Authors' own elaboration



(a)



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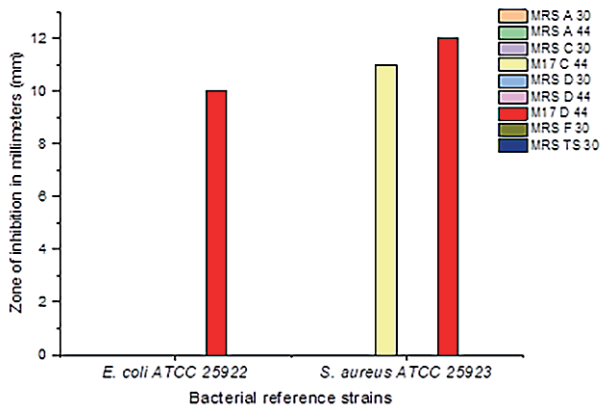
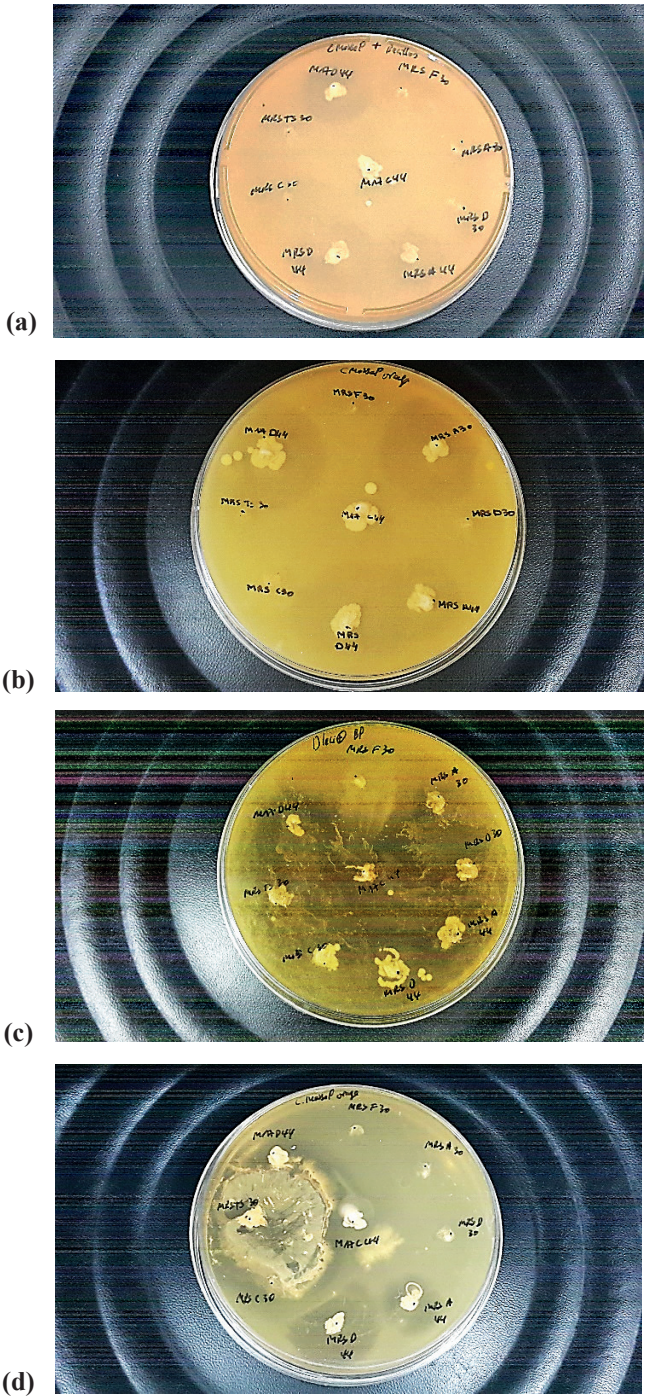


(c)

Fig. 8. Antibacterial effect of isolated LAB strains (a): Bacterial contaminants (Gram-negative bacteria); (b): Bacterial contaminants (Gram-positive bacteria); (c): Bacterial reference strains.

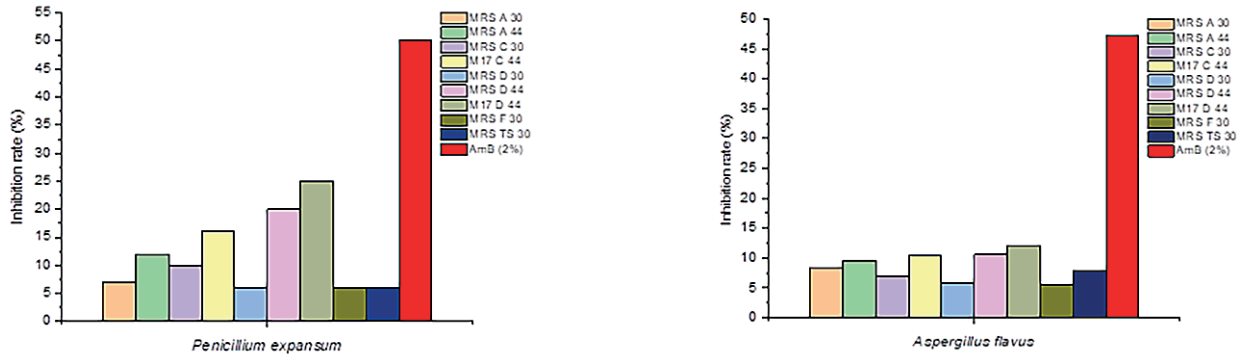
Source: Authors' own elaboration



(a) **(b)** **(c)** **(d)**

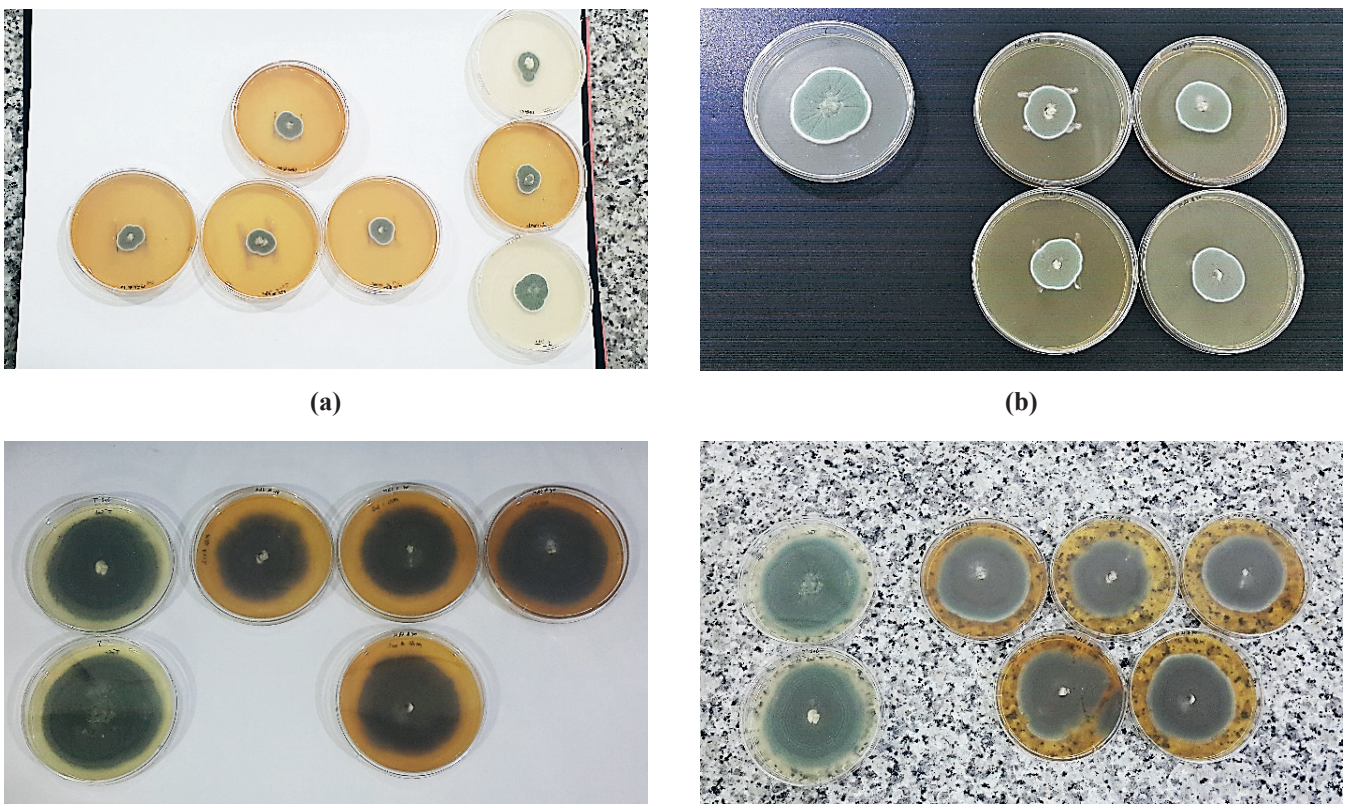
Fig. 9. Photographic illustration of the interaction between LAB strains and bacterial contaminants isolated from dried and salted camel meat (Kadid) (Original, 2024) (Photo: E. Benyagoub) (a, b, and c): *Klebsiella oxytoca*; (d): *Enterobacter sakazakii*.

Source: Authors' own elaboration



(a) **(b)**

Fig. 10. Qualitative antifungal effect of LAB strains against fungal contaminants isolated from dried and salted camel meat (Kadid) (Mycelial disc method)
(a): *Penicillium expansum*; (b): *Aspergillus flavus*; AmB (2%): Amphotericin B (2%).
Source: Authors' own elaboration



(a) **(b)**

(c) **(d)**

Fig. 11. Photographic illustration showing the interaction between LAB strains and molds isolated from dried and salted camel meat (Kadid) using the radial growth method (Original, 2024) (Photo: E. Benyagoub)
(a and b): *Penicillium expansum*; (c and d): *Aspergillus flavus*.
Source: Authors' own elaboration

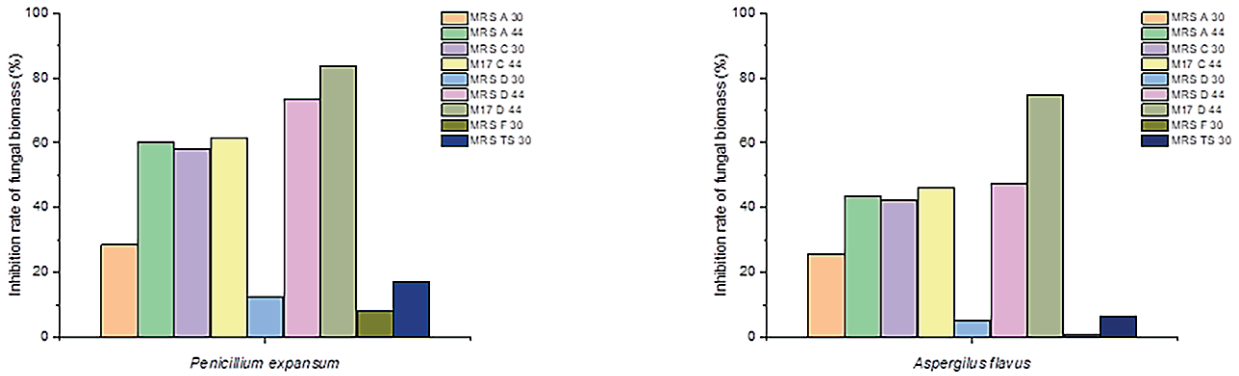


Fig. 12. Quantitative antifungal effect of LAB strains against fungal contaminants isolated from dried and salted camel meat (Kadid) (Fungal biomass method)
(a): *Penicillium expansum*; (b): *Aspergillus flavus*.
Source: Authors' own elaboration

The results showed that the isolated LAB strains (M17 D 44, MRS D 44, and M17 C 44) exhibited greater antibacterial activity compared to the other LAB strains, with zones of inhibition ranging from 10 mm to 40 mm.

Qualitative antifungal effect

The results of the qualitative antifungal effect of LAB strains against mold contaminants isolated from dried and salted camel meat (Kadid), assessed by measuring fungal radial growth, are presented in Figures 10 and 11.

The results showed that the isolated LAB strains exhibited a moderate to weak antifungal effect against *P. expansum* and *A. flavus* with inhibition rates ranging from 5% to 25% and 5% to 12%, respectively. In comparison, the control (AmB 2%) demonstrated high antifungal activity with inhibition rates of 50% and 47.3%, respectively. The maximum rate of fungal inhibition was attributed to the LAB strain M17 D 44, followed by MRS D 44, M17 C 44, and MRS A 44.

Quantitative antifungal effect

Figure 12 presents the result of the quantitative antifungal effect of LAB strains against *Penicillium expansum* and *Aspergillus flavus,* two fungal contaminants isolated from dried and salted camel meat (Kadid). The antifungal activity was measured by assessing the fungal biomass reduction.

The results showed that the LAB strains M17 D 44, MRS D 44, M17 C 44, MRS A 44, and MRS C 30 exhibited an antifungal effect against *P. expansum* and *A. flavus,* with inhibition rates ranging from 58% to 83% and 42% to 74%, respectively. In contrast, the other LAB strains were found to be less active, with inhibition rates ranging from 1% to 28%.

Conjugated antifungal effect

The combined antifungal effect of the LAB strains on fungal contaminants is shown in Figures 13 and 14.

The combination of LAB strains, both in binary and multiple forms, exhibited a stronger antifungal effect against *P. expansum* and *A. flavus* compared to individual LAB strains (> 28%; > 18% versus 5–25% and 5–12%, respectively). Additionally, it was observed that *A. flavus* was more resistant compared to *P. expansum*.

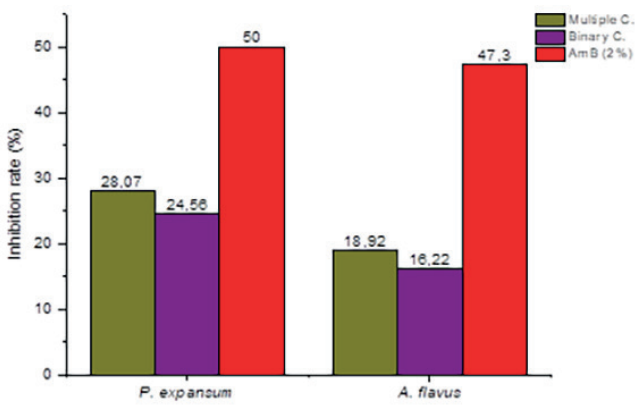


Fig. 13. Combined antifungal effect of LAB strains against *P. expansum* and *A. flavus* (Combination test – Mycelial disc method)
Multiple C: Multiple combined effect; Binary C: Binary combined effect; AmB (2%): Amphotericin B (2%).
Source: Authors' own elaboration

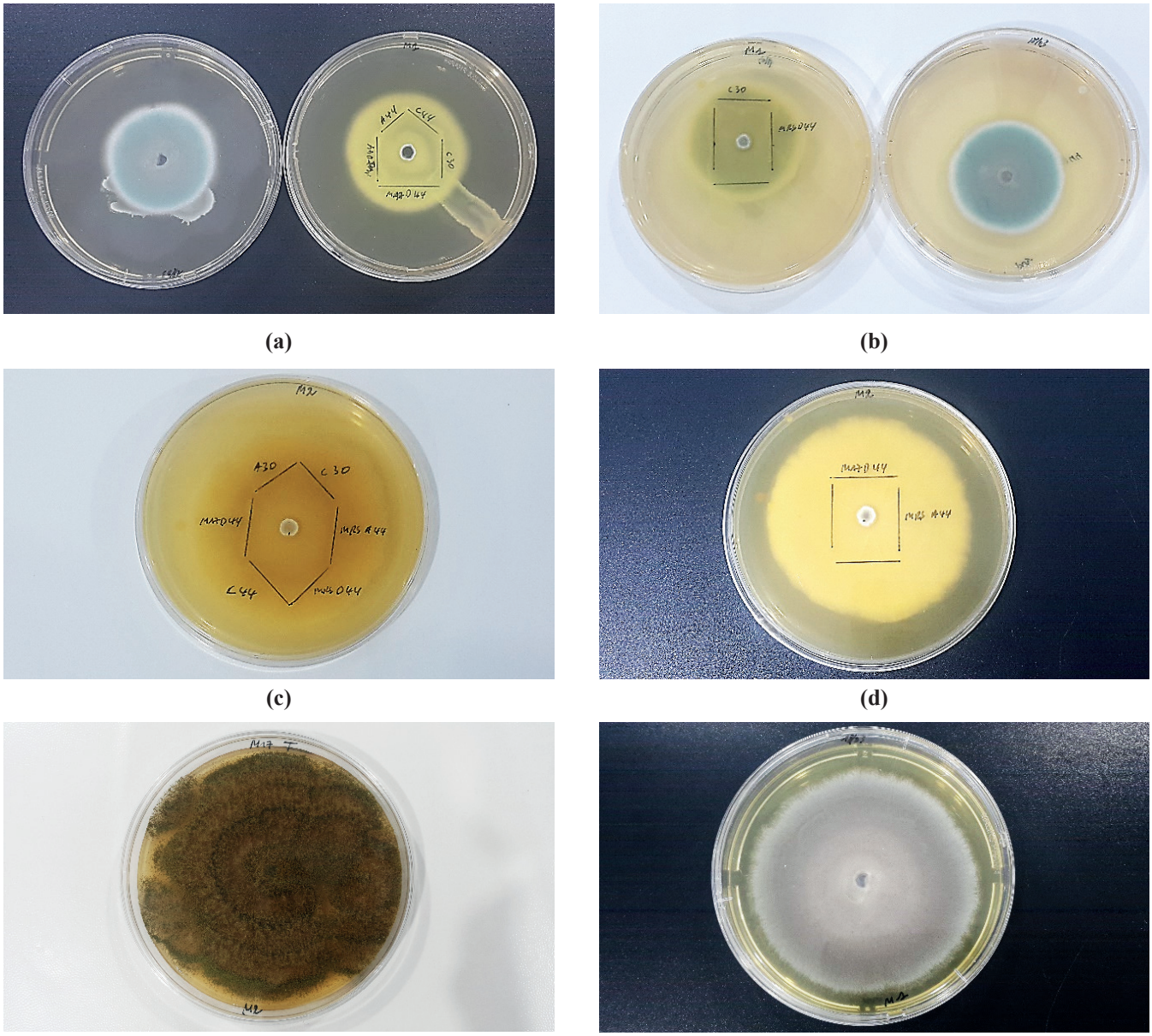


Fig. 14. Combined antifungal effect of LAB strains against *P. expansum* and *A. flavus* (Original, 2024) (Photo: E. Benyagoub)
(a): *Penicillium expansum* (Multiple combined antifungal effect); (b): *Penicillium expansum* (Binary combined antifungal effect); (c): *Aspergillus flavus* (Multiple combined antifungal effect); (d): *Aspergillus flavus* (Binary combined antifungal effect); (e): *Aspergillus flavus* (Control); (f): *Penicillium expansum* (Control).
Source: Authors' own elaboration

Discussion

In this study, we aimed to evaluate the antagonistic effects of lactic acid bacteria (LAB) isolated from dried and salted camel meat against certain microbial contaminants. We successfully isolated nine LAB strains from the dried and salted camel meat samples. The isolation of LAB strains from this type of dried, protein-rich food has been reported by several

authors, including Benlacheheb et al. (2019), Benaissa et al. (2022), Boubakri et al. (2022a), Benyagoub et al. (2022a), and Benyagoub et al. (*in press*). For example, Benaissa et al. (2022), reported that LAB strains isolated from camel meat belong to five genera: *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus*, and *Pediococcus*.

A total of 13 microbial contaminants were isolated from dried and salted camel meat, comprising both Gram-positive and Gram-negative bacteria, as well as two fungal species. Several studies have reported the isolation of enterobacteria, staphylococci, and fungal flora, with no detection of pathogenic bacteria such as *Salmonella* spp and *Listeria monocytogenes* (Ibrahim et al., 2017; Bader et al., 2021; Benyagoub and Mammeri, 2023; Benyagoub et al., 2024). However, Bader et al. (2021), Benlacheheb et al. (2019), Benyagoub and Mammeri (2023), and Benyagoub et al. (2024) noted the dominance of lactic acid bacteria and coagulase-negative staphylococci in Kadid samples.

Additionally, Tafinta et al. (2023) isolated four *Aspergillus* species classified as storage fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus,* and *Aspergillus terreus*) from dried meat samples sold in Sokoto Metropolis, Nigeria. Nummer et al. (2004), Benyagoub and Bessadet (2023), and Benyagoub et al. (2024) emphasize the importance of the Kadid preparation method as a safe preservation process against pathogenic bacteria, including *E. coli* O157, *Salmonella* sp, *S. aureus* and *L. monocytogenes*. However, the duration of air drying, as well as the handling of fresh meat and the addition of ingredients, remain primary sources of microbial contamination (Tafinta et al., 2023).

Reduced water activity from drying limits bacterial competition. Factors such as moisture, ambient temperature, storage duration, and pH can influence fungal growth and mycotoxin production in dried meat (Tafinta et al., 2023; Benyagoub and Mammeri, 2023), However, based on its physicochemical and microbiological characteristics, Kadid is considered a safe traditional meat product (Bader et al., 2021; Benyagoub and Mammeri, 2023; Benyagoub and Bessadet, 2023).

In this study, we found that cocci LAB strains produced higher titratable acidity, ranging from 2.5 to 3.5g/l, compared to that obtained by bacilli LAB strains. These results corroborate the findings of Saidi et al. (2019) and Benyagoub et al. (*in press*), suggesting that these lactic acid bacteria are good candidates for dairy fermentation. Furthermore, Saidi et al. (2019) and Boubakri et al. (2022b) reported that these bacteria are capable of inhibiting the growth of undesirable microorganisms and are also essential for flavor development.

According to Albenzio et al. (2001) and Fguiri et al. (2017), the acidification process of each LAB strain depends on its unique ability to break down and absorb nutrients in the medium, as well as the presence or absence of nutrient transport systems.

In this study, the results of antibiotic susceptibility testing for Gram-negative bacterial contaminants revealed resistance to penicillins and cephalosporins. These findings are consistent with our previous studies on bacterial contaminants isolated from various food matrices (Benyagoub et al., 2017; Benyagoub, 2022b; Razni et al., 2024). According to Zhao et al. (2001), Gram-negative bacteria possess a remarkable ability to acquire and accumulate antimicrobial resistance factors, often resulting in the expression of β-lactamases. Furthermore, food can act as a potential vector for the transmission of antibiotic resistance genes (Baskan and Celebi, 2024). This resistance is attributed to the production of enzymes such as beta-lactamases, penicillinases, and cephalosporinases, which inactivate these antibiotics, and/or to modifications in penicillin-binding proteins (PBPs). Additionally, clindamycin resistance is likely due to mutations in the genome. Moreover, the resistance of Gram-positive bacterial contaminants to penicillins is linked to the production of penicillinase enzymes and/or target modifications (Uddin et al., 2021; Benyagoub et al., 2021a; Benyagoub, 2024a). Due to their outer membrane, Gram-negative bacteria exhibit greater resistance compared to Gram-positive bacteria (Benyagoub et al., 2016; Benyagoub et al., 2020b; Benyagoub et al., 2020a; Benyagoub et al., 2021b; Synowiec et al., 2021).

Lactic acid bacteria are considered proteolytic if they possess a proteolytic system that includes proteinases, peptidases, and transport proteins (Kieliszek et al., 2021). According to Vuillemard (1986), a strain is considered proteolytic if it exhibits a lysis zone with a diameter greater than 5mm.

Saidi et al. (2019) reported that cocci LAB strains are frequently used as starter cultures due to their strong proteolytic activity. This aligns with our findings in this study and a previous one (Benyagoub et al., *in press*), where the cocci LAB strains – namely M17 D44, M17 C44, MRS D44, and MRS A44 – exhibited good proteolytic activity compared to other lactic acid bacteria.

For the antibacterial tests, the M17 D44 strain appears to be the most effective in inhibiting both Gram-positive and Gram-negative bacterial contaminants, with inhibition zones reaching up to 40mm. Bouzaid et al. (2016) reported that *Lactobacillus yamanashiensis*, isolated from ground camel meat, has a broader inhibitory power than other lactic acid strains. This strain was capable of inhibiting the growth of *Staphylococcus aureus, Citrobacter freundii, Proteus mirabilis, Pseudomonas fluorescens*, and three species of *Salmonella*. Additionally, Bouchefra et al. (2022) reported that the

diameters of the inhibition zones for bacteriocin-producing *Lactobacillus plantarum*, isolated from dried and salted camel meat, ranged between 11mm and 15mm when used to preserve goat cheese against *S. aureus*. The bacteriocin-producing lactobacilli isolated from meat samples exhibited antibacterial power against bacteria such as *Bacillus cereus, Salmonella typhi, Staphylococcus aureus, Escherichia coli,* and *Proteus vulgaris* (Zende et al., 2023). The data reported by Biscola et al. (2013) confirm the antibacterial effect of bacteriocin-producing *Lactococcus lactis* subsp. *lactis* strains isolated from Charqui, a Brazilian fermented, salted, and dried meat product, against *Listeria monocytogenes* and *Staphylococcus aureus*. These findings are consistent with our results.

In the same study, no activity was observed against the tested bacterial reference strains, namely *P. aeruginosa*, *E. coli*, and *B. cereus*. However, our LAB strains were active against these bacterial strains. This could be explained by the pronounced acidifying ability of the isolated LAB strains or the secretion of inhibitory substances (Stoianova et al., 2012). These results are supported by the study of Petit et al. (2014), which reported that dry biltong, a traditional salted and dried South African meat, exhibited a lower bacterial count and higher levels of both lactic acid bacteria and D-lactic acid compared to moist biltong.

Additionally, the absence of antimicrobial activity may be due not only to variations in the concentration of inhibitory substances produced by lactic acid bacteria (Benyagoub, 2022b), but also to factors such as temperature, pH, and the composition of the culture media (Bouzaid et al., 2016). Bacteriocins, as inhibitory substances, have been shown to be degraded by proteases produced by lactic acid bacteria. The emergence of resistance to bacteriocins from different classes in target bacteria has also been observed (Bouzaid et al., 2016).

According to Akhtar and Nawaz (2023), the yield of antimicrobial substances can be improved by providing the appropriate temperature and pH to promote the growth of lactic acid bacteria strain. This highlights the importance of the conditions and the techniques used to evaluate the antibacterial effect.

The tested LAB strains exhibited a moderate antifungal effect, with *Penicillium expansum* being more sensitive compared to *A. flavus* (5–25% vs. 5–10%). These results align with the findings reported by Sahel et al. (2022) and Benyagoub (2022b), who highlighted the antifungal effect of LAB strains isolated from camel and goat milk against fungal species such as *Penicillium spp* and *Aspergillus spp.* This effect restricts the radial growth of these fungal species by inhibiting conidia germination and/or fungal growth. The capacity of lactic acid bacteria (LAB) to suppress mycotoxigenic fungi varies based on the strain's ability to produce particular organic acids, which can differ between strains (Guimaraes et al., 2018).

The results of the antifungal effect using the quantitative method in broth medium showed significantly higher inhibition rates compared to the qualitative method on agar medium. *Penicillium expansum* was more susceptible to the tested LAB strains compared to *Aspergillus flavus* (10 – 80% vs. 5–70%). These findings are consistent with those reported by Sahel et al. (2022), who observed a fungal inhibition rate ranging from 8.82% to 80% against *P. expansum*.

The parameters that could influence the production of antifungal metabolites in the medium include the nature of the antimicrobial compounds, pH, incubation time and temperature, and the physical nature of the culture medium (agar or broth). Therefore, the variation in antifungal activity of LAB strains when using an agar medium may be related to the restricted diffusion of bioactive compound. The production and diffusion of inhibitory compounds tend to be more effective in a broth medium (Sahel et al., 2022; Benyagoub, 2022b; Benyagoub, 2023b; Wijenayake et al., 2022).

According to Nurmi et al. (1992), Stoianova et al. (2012), Siedler et al. (2019), Sahel et al. (2022), Bendada et al. (2022), Benyagoub (2022b), and Benyagoub (2023b), lactic acid bacteria (LAB) strains are known to produce antifungal compounds such as organic acids, diacetyl, bacteriocins, hydrogen peroxide, CO_2, reuterin, antifungal peptides, and to engage in competitive exclusion. These compound not only inhibit fungal growth but may also contribute to the detoxification of mycotoxins, including ochratoxins and aflatoxins. This property makes LAB strains promising candidates for use as food biopreservatives.

Conclusion

In this study, nine lactic acid bacteria (LAB) strains were isolated from dried and salted camel meat (Kadid). Most of these strains exhibited proteolytic potential (5 out of 9 LAB strains, i.e., 55.5%) and strong acidifying power, with lactic acid production ranging from 1.86 to 3.24 g/L. This may explain the antimicrobial activity results, as the LAB strains showed a more pronounced antagonistic effect against bacterial contaminants compared to their antifungal effect. Notably, *Penicillium expansum* was more susceptible to the tested LAB strains than *Aspergillus flavus*.

The LAB strains isolated from Kadid displayed significant technological and antimicrobial properties, making them suitable for use in the food industry as well as other applications.

Acknowledgment

No financial support was received for this study.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Received: **October, 20, 2024**; *Approved*: **May, 09, 2025**; *Published*: **August, 2026**