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Antifungal activity of lactic acid bacteria against *Aspergillus niger* and *Fusarium oxysporum*

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Abstract. Many fungi are viewed as contaminants in nearly all food products. This contamination can be affected by various pollutants, including natural toxins. Among these toxins are mycotoxins produced by mold pathogens that affect plants. Combating these pathogens is essential in the agri-food industry, and the development of innovative strategies like biopreservation presents a promising solution. This study aims to isolate lactic acid bacteria (LAB) from fermented cow's milk and examine their antifungal properties against *Aspergillus niger* and *Fusarium oxysporum*. The LAB were identified through morphological, biochemical, and MALDI-Tof analyses. Five strains of LAB sourced from fermented cow's milk were evaluated for their antifungal activity using both the streak method and the double-layer method. The LAB isolates displayed inhibitory effects against *Aspergillus niger* and *Fusarium oxysporum*, showing a significant reduction in mean fungal diameter in comparison to the control, with these isolates categorizing under the *Lactobacillus* genus. The *Fusarium oxysporum* strain exhibited greater sensitivity to LAB compared to the *Aspergillus niger* strain. No decrease in antifungal activity was noted after subjecting the inhibitory metabolites in LAB supernatants to temperature treatments (4°C, 45°C, 60°C, and 100°C). After treatment with the proteolytic enzyme (chymotrypsin), no alterations in inhibition zones were observed. Inhibition was noted at an acidic pH for all strains. Investigating the nature of the inhibitory metabolites

of LAB through thin-layer chromatography (TLC) and following their characterization allowed us to conclude that the antifungal properties of this LAB are attributed to the production of lactic and acetic acids.

Keywords: antifungal activity, biopreservation, lactic acid bacteria, MALDI-Tof, thin layer chromatography

Introduction

Microorganisms, especially lactic acid bacteria (LAB), are extensively employed in the food sector for fermentation procedures (Bourdichon *et al*., 2012). These bacteria play a crucial role in improving the quality of fermented items by creating distinct organoleptic features, such as flavor and texture, while maintaining the original taste and aroma of the product (Ledenbach, 2009).

Biopreservation entails the introduction of selected bacterial strains into a product to inhibit the growth of undesirable microbes, without affecting the product's organoleptic and health aspects. LAB are particularly ideal for this approach, as they often produce various inhibitory substances, including organic acids, hydrogen peroxide, diacetyl, bacteriocins, and reuterin, which exhibit antagonistic microbiological properties that hinder the proliferation of unwanted microbiota (Leyva *et al*., 2017), while preserving the organoleptic and health characteristics of the product.

Nonetheless, microbial contamination in food can trigger considerable physical and biochemical alterations, including the generation of mycotoxins, which pose significant health hazards and economic repercussions. To tackle this issue, biological control techniques utilizing natural compounds sourced from bacteria, fungi, or plants are being explored as eco-friendly methods to thwart mold proliferation and guarantee food safety (Heydari and Pessarakli, 2010). To combat microbial spoilage, food preservation strategies are primarily employed to avoid spoilage during storage and distribution, extending to consumer usage (Prokopov and Tanchev, 2007). These methods help to ensure that the anticipated shelf-life durations are upheld (Russell and Gould, 2003). Moreover, they inhibit the growth of bacteria and fungi in food, delay fat oxidation to minimize rancidity, and sustain the quality, such as color, texture, flavor, and nutritional content of food products. Some of the most prevalent techniques include drying (Rahman and Perera, 2007), cooling (Fennema, 1966), smoking (Lingbeck *et al*., 2014), vacuum packaging (Goulas and Kontominas, 2007), pasteurization (Steele, 2000), irradiation (Abdulmumeen *et al.,* 2012), and ultra-high temperature (UHT) treatment (De Alcântara *et al.,* 2022).

Various biological control techniques utilizing natural substances from bacteria, fungi, or plants are being explored as eco-friendly approaches to inhibit mold proliferation and guarantee food safety (Calpice and Fitzgerald, 1999). Among these techniques, LAB have been utilized in a range of food items, such as dairy (Erem *et al*., 2024; Jaafar *et al*., 2024), vegetables (Nemati *et al.,* 2023), and seafood (Elidrissi *et al.,* 2023), where they significantly contribute to prolonging shelf life. This application aligns with the growing consumer preference for minimally processed foods that do not contain chemical preservatives. In the dairy sector, LAB have been historically employed in the creation of fermented milk products and are classified as Generally Recognized as Safe (Amenu *et al*., 2023), underscoring their recognized safety and effectiveness. Numerous studies have been conducted to formulate antifungal cultures for the Bio preservation of dairy products (Batish *et al*., 1997).

The presence of mycotoxin-producing fungi in food not only presents a quality challenge for the global food industry but also leads to significant health risks due to the generation of various mycotoxins, some of which can be quite hazardous for food safety. In current large-scale food production systems, which encompass numerous processing stages and a variety of ingredients, fungal contamination is frequently unavoidable, even with adherence to good manufacturing practices (Sadiq *et al*., 2019). In today's society, health-conscious consumers prefer fresh and natural foods that do not contain synthetic preservatives or stabilizers. Mycotoxins are a major health hazard for consumers, making the search for effective methods to prevent or eliminate them a primary goal. LAB show promising potential in this area due to their resistance and functional capabilities. This work is organized based on that premise, aiming to underscore the antifungal properties of LAB (Siedler *et al*., 2019).

Molds present in food can be classified into two groups: beneficial molds, which are involved in fermentation and antibiotic production (such as *Penicillium roqueforti* and *Penicillium camemberti*), and harmful molds that lead to food spoilage. Mycotoxins, produced by the secondary metabolism of filamentous fungi or molds, result in mycotoxicosis when consumed by humans or animals (Bhatnagar *et al*., 2004). Although these toxic substances are generated by fungi, they are not critical for their growth. They may, however, function as a defense mechanism against other microorganisms in their surroundings. The primary mold genera implicated in mycotoxin production comprise *Aspergillus, Claviceps, Penicillium,* and *Fusarium* (Le Bars, 1998).

Various factors, including fungal species, climatic conditions, and agricultural practices for growing and storing products, affect mycotoxin levels in food (Castegnaro and Pofhl-Leszkowicz, 2002). The objective of the study was to explore the antifungal properties of LAB and to identify and characterize the

inhibitory metabolites contributing to this antifungal effect. The findings support the ongoing efforts to establish LAB as dependable bio-preservation agents in the food industry.

Materials and methods

Biological material

Lactic acid bacteria (LAB). LAB were isolated from fermented cow's mbarefoot ilk samples. These samples were collected directly from a farm located in Oran (western Algeria). Successive decimal dilutions were made in 9 ml of sterile physiological saline up to a dilution of about 10^{-6}, from which 1 ml was taken from the 10^{-4}, 10^{-5}, and 10^{-6} dilutions and inoculated into Petri dishes filled with MRS (deMan, Rogosa, and Sharpe) culture medium supplemented with $CaCO_3$ at 5 g/l. The cultures were incubated at 37°C for a period of 48 to 72 hours. After the incubation period, 10% of the various types of colonies that formed and had a clear halo were selected and purified on MRS, followed by further tests, including the catalase test and Gram staining. The identification of the LAB isolates was conducted using conventional analytical methods that relied on morphological examination, physiological and biochemical criteria, and were compared against the identification table (Carr *et al.,* 2002). Additionally, identification was performed through the MALDI-ToF method.

Phytopathogenic fungi. The fungal strains used in this study were primarily provided by the Saharan Natural Resources Laboratory at Adrar University, Algeria. The fungal species included: *Aspergillus niger* and *Fusarium oxysporum*. These fungal strains were subcultured on Potato Dextrose Agar (PDA) medium and incubated at 37°C for five days.

Identification of bacterial isolates using the Bioÿper Sirius GP MALDI system. The identification of bacterial isolates took place at the Genomics Technology Platform of the Oran School of Biological Sciences (Algeria) utilizing the MALDI Biotyper Sirius GP system from Bruker Daltonics, Germany.

Antifungal activity research. The antifungal activity of LAB against *Aspergillus niger* and *Fusarium oxysporum* was first evaluated through a qualitative test (Wang *et al*., 2012; Gerbaldo *et al.,* 2012), followed by a quantitative assessment (Barefoot and Klaenhammer, 1983).

Qualitative ("confrontation") test: Initially, two streaks of each lactic culture were applied on Petri dishes containing the MRS culture medium and incubated at 37°C for 48 hours. Subsequently, a 5mm disc of each fungus was positioned in the same dish, which was then incubated again at 30°C for three

days. After the incubation, the fungal growth diameter was measured and compared against a control, which consisted of an uninhibited fungal strain placed in the center of the dish containing PDA medium without LAB. The percentage inhibition of growth of the phytopathogenic fungus (I) was calculated using the following formula: $I = (Rw - Rt / Rw) \times 100$, where Rw represents the maximum radial distance of growth for the phytopathogenic fungus in the non-LAB control and Rt denotes the radial distance of growth for the phytopathogenic fungus towards the antagonist (measured in centimeters). All experiments were conducted in triplicate and repeated three times.

Quantitative (well) test: Wells were created in the agar surface of a Petri dish filled with MRS medium that had been inoculated with fungi, using a sterile tip. Subsequently, 100 µl of the supernatant from each culture was placed in the wells. The wells were formed with a cookie cutter on a Petri dish containing 10 ml of MRS, which was then overlaid with 10 ml of PDA medium that had a monosporal suspension (10^3 spores/ml); after this, 100 µl of the lactic bacteria supernatant was added to the wells. LAB strains were grown in MRS broth at 37°C, and after 18 hours of incubation, the cells were centrifuged at 4000 rpm for 15 minutes at 4°C. It's important to filter the supernatant through a 0.22 µm Millipore filter to inhibit bacterial cell growth. A negative control with no inoculated culture medium was established. After a 72-hour incubation at 37°C, the zones of inhibition around each well were evaluated.

Antifungal metabolite characterization

The temperature effect. The thermal influence on the crude active supernatant of LAB was examined at varying temperatures (4°C, 45°C, 60°C, 100°C) for a duration of 20 minutes (Teubeur, 1993; Stiles, 1996). Heating was performed in a water bath. This investigation was conducted using the Well Method (Barefoot and Klaenhammer, 1983).

The pH effect. Antifungal efficacy was evaluated in liquid MRS (medium deMan, Rogosa, and Sharpe) at pH levels of 2, 4, 6, and 8, followed by an 18-hour incubation at 37°C utilizing the well method described by Barefoot and Klaenhammer (1983).

Proteolytic enzyme effect. The influence of proteolytic enzymes was determined according to the methodology outlined by Hirsch, 1979. Accordingly, 10 µl of chymotrypsin enzyme (1 mg ml^{-1}, prepared in a buffer solution of 50 mM Tris-HCl, pH 8.0) was mixed with the active crude supernatant. The study utilized the previously mentioned well method (Barefoot and Klaenhammer, 1983). Zones of inhibition were evaluated after three days of incubation at 30°C and compared to the untreated control.

Identification of inhibiting metabolites

Thin layer chromatography (TLC). This method, which is based on adsorption, enables the initial separation and identification of metabolites in the supernatant, according to the approach described by Lee et al. (2001). The frontal ratio (Rf) is calculated as the ratio of the distance migrated by the sample (L1) to the distance traveled by the mobile phase (L2), serving as a comparative measure of metabolite migration. (Rf = L1/L2), where L1 represents the distance of the spot formation and L2 signifies the migration distance of the mobile phase.

Results

Identification of LAB

Following the purification of the various isolates on MRS agar medium, the LAB colonies appeared small, with a whitish or yellowish hue, transparent and smooth textures, and distinct lenticular shapes. The macroscopic observations after purifying the LAB isolates in liquid MRS medium displayed a clear halo on the surface, which signifies the microaerophilic characteristics of our strains. All test outcomes, determined by physiological and biochemical traits, are summarized (Tab. 1).

Table 1. Morphological, physiological and biochemical characteristics of lactic acid bacteria isolated from fermented cow's milk.

Properties	LAB1	LAB2	LAB3	LAB4	LAB5
Gram strain	+	+	+	+	+
Spores formation	-	-	-	-	-
Catalase activity	-	-	-	-	-
Fermentation type	Hetero-	Hetero-	Hetero-	Hetero-	Hetero-
Exopolysaccharide production	+/-	+/-	+/-	+/-	+/-
Use of citrates	-	-	-	-	-
Gelatin degradation	+	+	+	+	+
Acetoin degradation	-	-	-	-	-
Mannitol-mobility	-	-	-	-	-
Fermentation of D-fructose	+	+	+	+	+
D-Mannose	+	+	+	+	+
D-Raffinose	+	+	+	+	+
Saccharose	+	+	+	+	+
Ribose	+	+	+	+	+

Properties	LAB1	LAB2	LAB3	LAB4	LAB5
Gluconate	+	+	+	+	+
Lactose	+	+	+	+	+
Galactose	+	+	+	+	+
Esculine	+	+	+	+	+
D-Glucose	+	+	+	+	+
Maltose	+	+	+	+	+
D-tagatose	+	+	+	+	+
Growth at 15°C	-	-	-	-	-
Growth at 45°C	+	+	+	+	+
ADH degradation	-	-	-	-	-

Identification of bacterial isolates using the MALDI-TOF Biotyper Sirius GP system

Following a series of triplicate analyses conducted on the samples utilizing the MALDI-TOF BIOTYPER Sirius GP system, all five strains were identified as *Limosilactobacillus fermentum*, achieving a high overall confidence score (Tab. 2). The findings suggest that *Limosilactobacillus fermentum* is predominant in the ecological niche from which the samples were isolated. Additionally, there is a potential for redundancy of the same strain. To validate this assumption, further investigations, such as REP-PCR and 16S rRNA sequencing, should be performed.

Table 2. Identification of isolated strains by the MALDI-TOF/BS GP method with the log value of similarities.

Strain code	Matched pattern	Score
LAB1	*Limosilactobacillus fermentum* DSM 20391 DSM-2	2.16
LAB2	*Limosilactobacillus fermentum* 21 –PG -1 ZZMK	2.05
LAB3	*Limosilactobacillus fermentum* 21 –PG -1 ZZMK	2.10
LAB4	*Limosilactobacillus fermentum* 21 –PG -1 ZZMK	2.08
LAB5	*Limosilactobacillus fermentum* 21 –PG -1 ZZMK	1.86

Study of the antifungal activity of LAB

The study examined the production of antifungal agents targeting *Aspergillus niger* and *Fusarium oxysporum* using the streak method outlined by Magnusson *et al.* (2003), which facilitated an initial selection of LAB with anti-*Aspergillus* and anti-*Fusarium* properties. The direct confrontation tests between LAB and phytopathogenic fungi indicated a significant decrease in fungal diameter in the presence of LAB compared to the control group.

The inhibition effects appeared to vary by strain, with the *Fusarium* strain demonstrating greater sensitivity to the LAB supernatant than the *Aspergillus* strain. The assessment disclosed a range of antifungal effectiveness among the bacterial strains, with fungal growth inhibition rates varying from 33% to 100%. LAB2 exhibited low antifungal efficacy against *Aspergillus niger*, while LAB1 and LAB5 showed moderate inhibitory effects, stopping the fungus's growth, in contrast to LAB3 and LAB4, which demonstrated strong activity against *Aspergillus*. The findings indicate that certain strains of LAB possess significant inhibitory action against *Fusarium*, leading to a total lack of fungal growth (Fig. 1, 2).

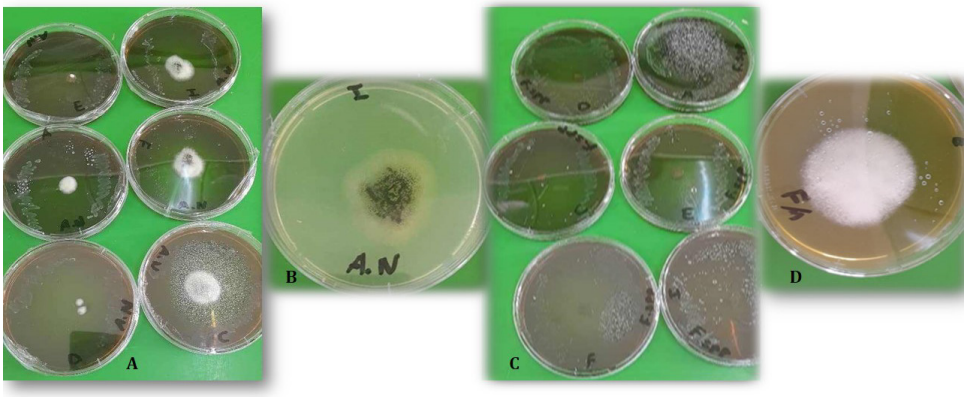

Figure 1. A: Antifungal activity of strains of LAB against *Aspergillus niger* by the streak method. B: *Aspergillus niger* control. C: Antifungal activity of strains of LAB against *Fusarium oxysporum* by the streak method. D: *Fusarium oxysporum* control.

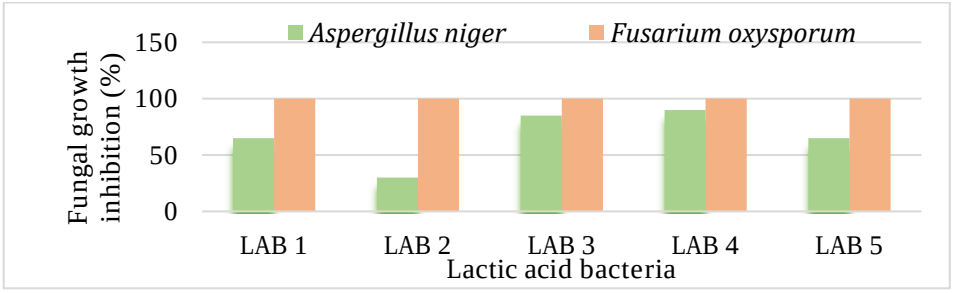

Figure 2. Inhibition of two phytopathogenic strains (*Aspergillus niger* and *Fusarium oxysporum*) by 05 strains of lactic bacteria. LAB1: *Limosilactobacillus fermentum* DSM 20391 DSM-2(2.16), LAB2: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.05, LAB3: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.10, LAB4: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.08, LAB5: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 1.86

Characterization of antifungal metabolites

The areas of inhibition differ based on how the inhibiting metabolites in the supernatants are treated.

The temperature effect. No decrease in antifungal effectiveness was noted after exposing LAB metabolites to temperatures of 4, 45, 60, and 100 °C for 20 minutes. This compound was observed to maintain its stability for 20 minutes across the temperature range of 4°C to 100°C. The inhibitory compound generated by lactic isolates is regarded as thermally stable (Fig. 3, 4).

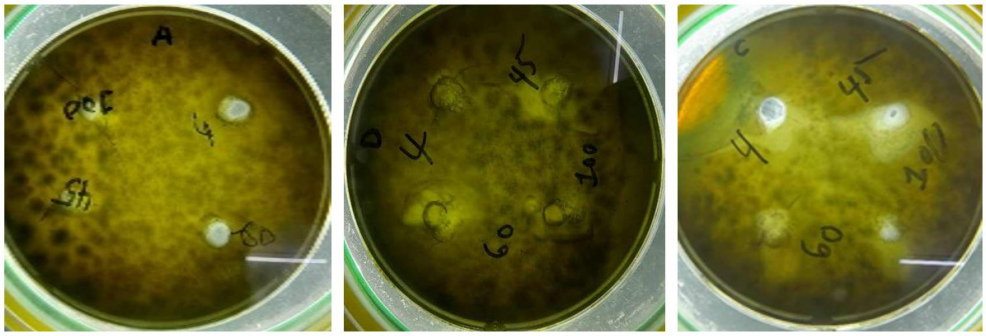

Figure 3. The effect of different temperatures on antifungal substances produced by LAB against *Fusarium oxysporum*.

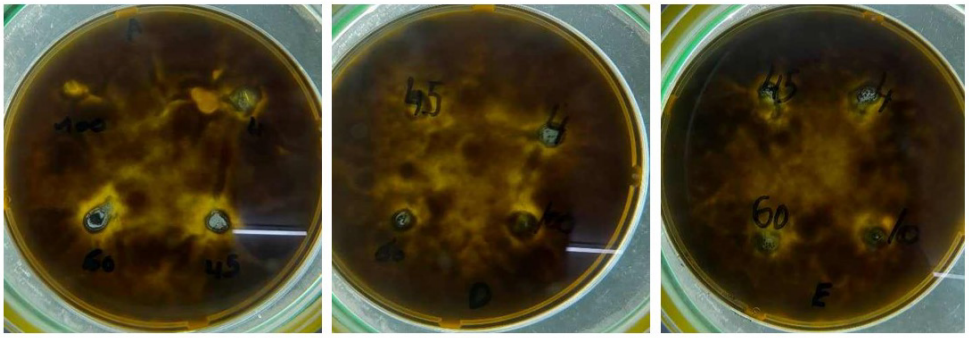

Figure 4. The effect of different temperatures on antifungal substances produced by against *Aspergillus niger*.

The pH effect. The findings indicated that the antifungal effectiveness of the supernatant diminishes as the pH increases, while a drop in pH corresponds with an enhancement in this activity, peaking at pH 2, 4, and 6 against *Aspergillus*

niger and *Fusarium oxysporum*, decreasing once pH reaches 8 (Fig. 5). This trend is frequently seen in antifungal agents, where pH significantly impacts the stability and effectiveness of the active ingredients. Additionally, we tested an SRM medium buffered to pH 7 to determine if there would be any suppression of fungal growth, with the results showing antifungal activity at neutral pH.

Proteolytic enzyme effect. Treatment of antifungal substances with the proteolytic enzyme (Chymotrypsin) does not appear to alter their inhibitory activity. This finding suggests that the enzyme's action on these compounds does not compromise their ability to inhibit fungal growth, offering a promising new avenue in the development of potential antifungal treatment methods, which suggests that the antifungal activity may be due to compounds other than proteins.

Research of the nature of antifungal substances. This initial study regarding the biochemical properties of the metabolites generated by LAB indicated that these substances are resistant to heat, maintain their efficacy when exposed to chymotrypsin treatment, and are more potent at acidic pH levels, implying a significant role of organic acids in their antifungal action.

Thin layer chromatography (TLC)

The retention factors (Rf) determined with a mobile phase migration distance (L2) of 7 cm show that acetic organic acids have a better migration performance, while lactic acid exhibits a shorter migration distance (Tab. 3, Fig. 5).

Table 3. Frontal ratios of organic acid revelation spots.

Depots	L2	RF=L1/L2
Lactic acid	0.5	0.071
Acetic acid	2.5	0.35
Citric acid	3.5	0.5
Limosilactobacillus fermentum DSM 20391 DSM-2 (2.16)	2.5	0.35
Limosilactobacillus fermentum 21-PG-1 ZZMK 2.05	2.5	0.35
Limosilactobacillus fermentum 21-PG-1 ZZMK 2.10	2.5	0.35
Limosilactobacillus fermentum 21-PG-1 ZZMK 2.08	2.5	0.35
Limosilactobacillus fermentum 21-PG-1 ZZMK 1.86	0.5	0.071

Figure 5. Separation of organic acids by thin-layer chromatography (TLC). AL: lactic acid, AC: citric acid, AA: acetic acid, LAB1: *Limosilactobacillus fermentum* DSM 20391 DSM-2(2.16), LAB2*: Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.05, LAB3: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.10, LAB4: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 2.08, LAB5: *Limosilactobacillus fermentum* 21-PG-1 ZZMK 1.86.

The supernatant from most of the strains exhibited an Rf value of 0.35, which corresponds precisely to that of acetic acid; thus, we can conclude that in the various strains where inhibition was observed, acetic acid is the predominant organic acid present. Conversely, one specific strain's supernatant showed an Rf value of 0.071, matching that of lactic acid. This study reinforces the notion that the antifungal properties of LAB against *Aspergillus niger* and *Fusarium oxysporum* are attributed to the organic acids: acetic and lactic.

Discussion

LAB isolates were characterized through two different stages. The initial stage involves Gram staining, catalase activity testing, and spore identification. The second stage focuses on both macroscopic and microscopic morphological examinations, alongside fermentation type analysis. Based on identification using the MALDI-Tof method, all lactic bacteria from fermented cow's milk selected from the Oran region are classified under the genus *Limosilactobacillus*. As noted by Sudeepa and Bhavini (2020), *Lactobacillus fermentum* is a species within the *Lactobacillus* genus known for its wide-ranging probiotic and antimicrobial characteristics, as well as its straightforward cultivation and characterization. Exploiting this bacterium for meaningful applications that could benefit humanity offers a valuable opportunity.

LAB demonstrate significant antagonistic properties against various microorganisms, including pathogens and organisms that cause food spoilage. To address the germs linked to food poisoning, eight strains of *lactobacilli* were isolated and identified from raw goat milk in western Algeria, primarily belonging to the following dominant species*: Lb. plantarum* (*Lb.*58), *Lb. plantarum* (*Lb.*68), *Lb. casei* (*Lb.*13), *Lb. rhamnosus (Lb.*54 and *Lb.*52), *Lb. paracasei* subsp. *paracasei* (*Lb.*55), *Lb. sakei* subsp. *sakei* (*Lb.*21), and *Lb. plantarum* (*Lb.*22) (Mami, 2013).

The findings of this research indicate that the antifungal properties of strains of *Lactobacillus* spp exhibit varying levels of antifungal efficacy against *Aspergillus niger* and *Fusarium oxysporum*. Additionally, although all five strains belong to the same genus, their zones of inhibition differ. The inhibitory effects are strain-dependent, with *Fusarium* strains appearing to be more susceptible to the effects of LAB supernatant compared to *Aspergillus* strains. Comparable findings were reported by Nazareth *et al.* (2019), who observed that *L. plantarum* CECT 748 and *L. plantarum* CECT 749 were the only ones demonstrating antifungal activity against all *Fusarium* and *Aspergillus* strains. Further investigations conducted by Guo *et al.* (2012) revealed that various *Lactobacillus* strains exhibit strong antifungal effects against *A. fumigatus* and *A. niger.*

Additional studies by Dalie *et al.* (2010) indicated a correlation between cell growth, pH levels, and the generation of antifungal metabolites. These metabolites retain their antifungal properties even after being subjected to 120°C for 20 minutes. However, research by Hansal *et al.* (2024) indicated that *Leuconostoc* selected against Gram-positive indicator bacteria showed a decrease after heat treatments (60°C/30 min, 80°C/15 min, 100°C/15 min), with most strains completely vanishing after 120°C for 10 minutes. Batish *et al.* (1997) and Sathe *et al.* (2007) proposed that pH is a critical factor in the synthesis of antifungal metabolites, with optimal production seen in *Lc. lactis* subsp. *diacetylactis* at pH levels of 6 and 8. Research has demonstrated that pH fluctuations can affect solubility, bioavailability, and the interaction between antifungal compounds and fungal cell membranes, thereby influencing their overall effectiveness. The application of proteolytic enzyme (Chymotrypsin) to antifungal substances does not seem to impact their inhibitory performance. Similar findings were reported by Laref (2014).

The current study validates that LAB exhibit antifungal properties against *Aspergillus niger* and *Fusarium oxysporum* due to the presence of acetic and lactic acids. As noted by Riley and Wertz (2002), the primary factor behind the inhibitory action of LAB is organic acids. Moreover, Sadiq *et al.* (2019) indicate that lactic, acetic, and propionic acids can slow the growth of *Aspergillus niger*, *Penicillium corylophilum*, and *Eurotium repens.* These acids interact with the cytoplasmic membrane, leading to the disruption of the membrane potential

and hindering active transport mechanisms. *Lactobacillus* species are well established for their production of organic acids in culture environments and for their capacity to generate hydrogen peroxide and other antimicrobial substances (Barefoot and Klaenhammer, 1983). The inhibitory effects of *Lactobacilli* may stem from the production of lactic and/or acetic acid; indeed, lactobacilli are recognized for their notable resistance to acidic conditions (Wilson *et al.,* 2005; Benthin and Villadsen, 1995), in addition to the synthesis of bacteriocins (Lrsen *et al*., 1993; Avila *et al.,* 2005).

Conclusions

The objective of the current study was to explore the antifungal properties of lactic bacteria in relation to *Aspergillus niger and Fusarium oxysporum*, as well as to identify the inhibitory metabolites involved. Five bacterial isolates were obtained from fermented cow's milk and underwent additional analysis. These isolates were recognized as LAB through morphological, biochemical, and physiological assessments, along with MALDI-Tof identification.

Following the qualitative and quantitative assessment of the inhibitory effects of the lactic isolates, it was observed that these strains exhibited significant fungal inhibition. It seems that the inhibitory activity differs among the various strains. The strains of *Fusarium oxysporum* demonstrated greater sensitivity to the impacts of the supernatants from lactic bacteria compared to *Aspergillus niger* strains.

The compounds produced by LAB are stable against temperature changes and proteolytic enzymes, exhibiting strong antifungal properties at acidic pH levels. The use of thin-layer chromatography (TLC) has confirmed that the antifungal action of LAB against *Aspergillus niger* and *Fusarium oxysporum* is attributed to the production of organic acids like acetic and lactic acids. The inhibitory mechanism of LAB is intricate, necessitating further investigation to conclusively establish their antifungal effectiveness, safety, and biocompatibility.

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