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Characterization and antimicrobial potential of lactic acid bacteria isolated from fermented figs and wheat against pathogenic bacteria

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ABSTRACT

This study focuses on the isolation and antimicrobial activity screening of lactic acid bacteria (LAB) strains from fermented wheat "Hamoum" and figs soaked in olive oil, both traditional products from western Algeria, as well as their ability to produce bacteriocins. The use of these LAB strains, derived from natural and local sources, represents an eco-friendly and safe alternative to conventional chemical preservatives in the fight against pathogenic bacteria. The antimicrobial activity of LAB isolates against both Gram-positive and Gram-negative pathogens (*Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Listeria monocytogenes*, and *Listeria innocua*) was evaluated using the well diffusion method on soft agar. Treatment of the inhibitory substances with proteolytic enzymes (proteinase K and trypsin) confirmed their proteinaceous nature, indicating they are likely bacteriocins. These molecules also exhibited remarkable stability under extreme pH (pH 2) and temperature conditions (4 °C and 80 °C), making them suitable for industrial applications. Furthermore, EDTA, Tween 80, and H_2O_2 were found to enhance the antimicrobial activity, while SDS and Triton X-100 partially inhibited it. Due to their strong activity against Listeria species and physicochemical stability, these bacteriocins are classified as class IIa, making them promising candidates for use as natural biopreservatives.

Keywords: lactic acid bacteria, bacteriocins, antimicrobial activity, pathogenic bacteria, natural preservatives, listeria, class IIa bacteriocins.

INTRODUCTION

Fermentation plays a fundamental role in the sustainable and economical preservation of food, directly contributing to global food security (Savary-Auzeloux and Rul, 2021). Microorganisms present in fermented foods act as natural "micro-factories", producing nutrients and bioactive compounds beneficial to human health, while reducing the reliance on chemical additives (Xiang et *al*., 2019).

In Algeria, a traditional and emblematic example of this practice is the use of fermented wheat, known as "hamoum", in the preparation of couscous. This wheat undergoes spontaneous fermentation in underground silos called Matmoras, where microorganisms naturally transform the wheat, enabling its preservation under environmentally friendly conditions (Mehel et *al*., 2019).

Similarly, Mediterranean products such as olive oil and figs are recognized not only for their nutritional benefits but also for their ecological value as local plant resources rich in bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties (Zarrouk et *al*., 2008; Selka et *al*., 2019).

Lactic acid bacteria (LAB) are central to natural fermentation processes. They participate in the transformation of various plant- or animal-based raw materials, while ensuring ecological valorization of local resources. Their ability to produce bacteriocins naturally occurring antimicrobial compounds offers a sustainable and eco-responsible alternative to chemical preservatives, allowing for effective and safe food preservation (Savary-Auzeloux and Rul, 2021).

Recognized by the FAO/WHO since 2002, bacteriocins produced by LAB have gained increasing interest in the field of food safety as they limit the use of synthetic and often polluting products, while extending the shelf life of foods.

Thus, this study aims to isolate lactic acid bacteria strains from traditional fermented products to select those capable of producing bacteriocins effective against pathogenic strains. Strain identification will be performed using the API 50 CHL gallery, and the study will also focus on the physicochemical parameters favorable to optimal production of these antimicrobial compounds.

MATERIALS AND METHODS

Pathogenic bacteria and medium used

The pathogenic strains that represent Gram negative bacteria used in this study were Pseudomonas aeruginosa ATCC27853 and *E. coli* ATCC25922 and for Gram-positive bacteria, the indicator strains *Staphylococcus aureus* ATCC 25923, Listeria monocytogenes ATCC19115, and Listeria innocua ATCC 33090 were used. The nutrient broth medium was used to inoculate the pathogenic bacteria cited above.

Isolation of lactic acid bacteria

The isolation of lactic acid bacteria was carried out from fermented wheat called "Hamoum" originating from southern Algeria and dried figs preserved in olive oil from the western part of the country.

1.0 g of each food was added separately to 9 mL of 0.9% sterile saline (NaCl), then 1 mL of this sample was sequentially diluted 104 to 107 times using 0.9% sterile saline. After that, 100 µL of each diluted sample was innoculated on sterile MRS agar and incubated at 37 °C for 24–48 h at anaerobic condition. Colonies with the typical characteristics of lactic acid bacteria were picked (Cappello et *al*., 2023) and successively propagated until we obtained single colonies. The purified single colonies were Gram stained (Moyes et *al*, 2009).

Single colonies were suspended on MRS broth, then V/V of 50% glycerol was added and the tubes were placed into a –20 °C freezer for storage.

Screening of antimicrobial-producing lactic acid bacteria

The agar well diffusion method was used to evaluate the antimicrobial capacity of LAB as described by Schillinger and Lücke (1989). Briefly, the LAB isolates were grown in buffered MRS broth at 37 °C for 18 h (Benmouna et *al*., 2018), the culture was centrifuged at 12,000 rpm for 15 minutes. The NA was cooled to approximately 40–50 °C, and the pathogens were inoculated at a concentration of 1% (v/v), thoroughly mixed, and then poured into Petri dishes.

After spreading 0.1 mL of overnight grown cultures from the pathogenic bacteria on the agar plates by soft agar overlay, wells of 6 mm diameters were punched. Aliquots (50 µL) from the supernatants of the LAB cultures were dispensed into the wells.

The diffusion was carried out at 4 °C overnight, followed by incubation at 37 °C (optimal temperature for the growth of the aforementioned pathogenic bacteria) for 24 to 48 hours. After which the zone of the inhibition was measured. The diameter of clear inhibition zone around the wells was measured with a ruler and isolates were rated as –, +, ++ and +++ (–: 0.6 cm; +: >0.6-0.9 cm; ++: 0.9-1.5 cm and +++: >1.5 cm) (Keresztény et *al*., 2024). The strain exhibiting the most prominent antimicrobial activity was chosen for use in subsequent experiments.

Bacteriocin activity test

The nature of the inhibitory molecules was investigated as described by Benmouna et *al*. (2020) with some modification. The LAB strains were inoculated in buffered MRS, at 2% of inoculum, and incubated for 20 h at 37 °C. The CSF were treated with the proteolytic enzymes: proteinase K and trypsin at 1 mg/ml for 2 h at 37 °C. Then, the samples were boiled for 5 min in order to inactivate the enzymes. The treated and untreated (without enzymes) were tested by the well diffusion test as described.

Effect pH and temperature on antimicrobial activity

The CFS were adjusted to pH values with sterile 1 N NaOH or HCl to achieve pH values of 2, 4, 6, 8, and 10. All of samples were tested for antimicrobial activity using the well diffusion method (Benmouna et *al*. 2018).

50 µl of CFS were incubated at various temperatures: 30 °C and 45 °C for one hour, 4 °C for 96 hours, 80 °C for 30 minutes, and at 120 °C for 20 min (autoclaving condition). The samples were tested by the method described by Schillinger and Lücke in 1989.

Effect of anionic, denaturing, and surfactant agents on antimicrobial activity

To evaluate the stability of the antimicrobial activity of the bacteriocin in the presence of various chemical agents, equal volumes of supernatant were treated with the following compounds: SDS (1%), Tween 80, urea, NaCl (6%), Triton X-100, EDTA (0.01 mM), and H_2O_2.

Each mixture was tested for its inhibitory activity using the agar diffusion method.In parallel, to verify the individual effect of each compound on the indicator bacterium, distilled water containing the same concentrations of the agents was tested separately, following the methodology of Albano et al. (2007).

Statistical analysis

Statistical analyses were performed using Python 3 and the Jupyter environment. The Shapiro-Wilk test was used to assess the normality of the data, while Levene's test evaluated the homogeneity of variances. Analysis of variance (ANOVA), followed by Tukey's post-hoc test, was applied to compare the effects of pH, temperature, as well as various surfactants, denaturing agents, and ionic agents on antimicrobial activity.

P-values were included to support the robustness of the results, with a significance threshold set at 5% ($p < 0.05$), ensuring the reliability of the conclusions.

The objective of this study was to evaluate whether the tested physicochemical parameters significantly influenced the antimicrobial activity of the isolates. For this purpose, ANOVA was used to detect any statistically significant differences between groups. When a significant effect was observed, Tukey's post-hoc test was conducted to precisely identify which group comparisons showed significant differences.

Identification of bacteriocin-producing strains

The two strains with the highest antibacterial activity were selected for species identification using the API 50 CH (bioMerieux, Lyon, France).

The identification of LAB strains was carried according to the instructions of the manufacturer. These results are then translated into a numerical profile based on the observed reactions. The identification of the bacteria is done using a binary system (0 and 1), according to the presence or absence of each metabolic reaction. The obtained profile is compared to the API database to identify the strain.

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria

Based on the typical morphological characteristics of LAB, a total of nine strains were isolated. The rod-shaped cells isolated bacteria were potentially identified as Lactobacillus and were chosen for further study.

The nine Lactobacillus strains isolated from Hamoum were coded as H1-1, H1-2, H2-1, H2-2,

H3-1 and H3-2) and from figs were coded as F1-2, F2-1, F2-2 (Figure 1 (a, b)).

The 9 strains with the largest antimicrobial inhibition zones are displayed in Table 1. The strains H3-1, H1-1, H2-2, and F1-2 exhibited the largest diameter of inhibition zone against *Listeria innocua*. The strains H1-1, H2-1, H3-1 and F2-1 also showed a larger inhibition zone against *Staphylococcus aureus*. However, no inhibition was observed against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* by all the LAB strains. The strains appeared purple after Gram staining, catalase-negative, able to grow under anaerobic conditions and exhibited coccoid and rod-shaped cells without spores when observed under a microscope (Figure 2).

The buffered medium stabilizes the pH of the medium and attenuates the acidity produced by lactic acid bacteria (Labioui et *al*., 2005); clarification is therefore due to inhibitory molecules.

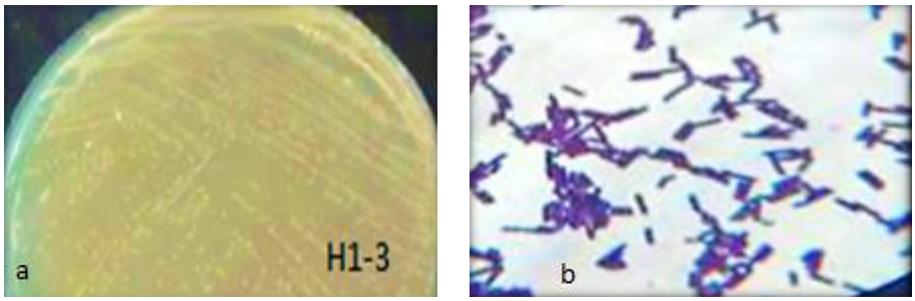


Figure 1. Morphological and Gram staining of isolated strains. (a): colony morphology of lactic acid bacteria in MRS agar. (b): Gram staining of lactic acid bacteria. Cells are purple, rod-shaped, and without spores

These molecules may include bacteriocins, organic acids or other antimicrobial metabolites, which inhibit the growth of surrounding pathogens, leading to the formation of clarification zones around the wells.

The tested strains exhibit significant inhibitory activity against *Listeria innocua*, but remain inactive against the pathogens *Pseudomonas aeruginosa* and *Escherichia coli*. These results are similar to those obtained by Tahlaiti (2019), who found that several *Lactobacillus* isolates cannot inhibit these two pathogenic microorganisms.

No inhibitory activity was detected against *Listeria monocytogenes* in the tested strains, unlike the findings of Garcia-Lopez et al. (2023), who reported inhibitory activity of their *Lactobacillus paraplantarum* BPF2 bacteriocins against this pathogen. These results are consistent with those of Hartmann et *al.* (2011), who found that after a spot diffusion test on agar medium, *Lb. plantarum* DSM1055 had no inhibitory effect on *Listeria monocytogenes* DSM20600.

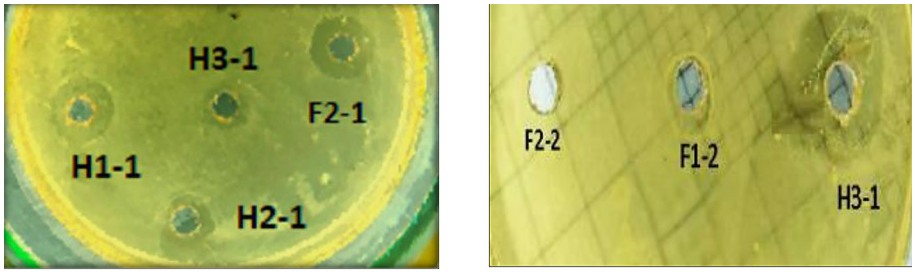
The results obtained reveal an inhibitory activity of strain H3-1 against *Staphylococcus aureus*, surpassing that observed by Tahlaiti (2019), where the *Lb. plantarum* BHL23 strain showed only moderate inhibition.

Due to their high inhibitory activity against *Listeria innocua*, the five strains H3-1,F2-1, H1-1, H2-1and H2-2 as well as the pathogenic strain, are selected for the continuation of the study.

Nature of the inhibitory molecules

Both bacteriocins were completely inactivated after treatment with the proteolytic enzymes trypsin and proteinase K, an example of the results for strains F2-1 and H3-1 is presented in Figure 3, while in Table 2 summarizes the results for all nine strains.

This indicates that the molecules responsible for the inhibitory activity are proteinaceous in nature, likely bacteriocins. Proteinase K and trypsin are proteolytic enzymes that degrade proteins, and the absence of inhibitory activity after their addition suggests that the active compounds are peptides or proteins. This observation is consistent with the characteristics of bacteriocins, which are antimicrobial peptides produced by bacteria to inhibit the growth of other closely related bacteria. The proteinaceous nature of bacteriocins has been widely confirmed by studies showing that these peptides are sensitive to degradation by proteases such as trypsin and proteinase K (Gänzle et *al*.,



Staphylococcus aureus ATCC 25923 *Listeria innocua* ATCC33090

Figure 2. The inhibitory effect of antimicrobial compounds produced from LAB H1-1, H2-1 isolates against *Staphylococcus aureus* ATCC25923 and LAB H3-1, F1-2 isolates on *Listeria innocua* ATCC33090

Table 1. Measurement of inhibition zones of lactic acid bacteria strains against pathogenic strains

Indicator strains	Inhibitory activity against pathogenic bacteria								
	H1-1	H1-2	H2-1	H2-2	H3-1	H3-2	F2-1	F1-2	F2-2
Listeria innocua ATCC33090	++	+	++	++	+++	-	+++	+	-
Listeria monocytogenes ATCC19115	-	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 25923	++	-	++	-	++	-	++	-	-
Escherichia coli ATCC25922	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC27853	-	-	-	-	-	-	-	-	-

Figure 3. Effect of proteinase K and trypsin on the inhibitory activity of the two lactic acid bacteria strains F2-1 and H3-1. (1: H3-1 untreated, 2: F2-1 untreated, 3: H3-1 treated with proteinase K, 4: F2-1 treated with proteinase K, 5: H3-1 treated with trypsin, 6: F1-2 treated with trypsin)

2000; Drider et al., 2006). These antimicrobial peptides are often produced during fermentation and have shown a broad spectrum of activity against foodborne pathogens (Hammes and Hertel, 2009; Parvez, 2017).

Study of physicochemical parameters affecting bacteriocin activity

The results obtained for the different tested physicochemical parameters indicate that the data related to pH, temperature, anionic agents, denaturing agents, and surfactants follow a normal distribution and exhibit homogeneity of variances. Therefore, ANOVA was applied to determine whether there are statistically significant differences among the groups corresponding to these physicochemical parameters.

Effect of pH

The exposure of bacteriocins to different pH values showed that both remained fully active in the pH range of 2–6. Reduced activity of both bacteriocins was found after treatment at pH 8 and pH 10 (Figure 4). An example of the results is shown in Figure 5. The results of the statistical analysis are summarized in the Tables 3 and 4.

Following the ANOVA, we obtained an F-ratio of 106.42 with a p-value less than 0.0001. These results indicate a statistically significant difference between the pH groups. The table below presents the results of the Tukey post-hoc test.

The results obtained indicate that there are significant differences between most pairs of pH groups, with the exception of the comparisons between pH 2 vs pH 6 (p-adj = 0.2965) and pH 10 vs pH 8 (p-adj = 0.9984), for which the adjusted p-values are not significant ($p > 0.05$). This suggests that there is no statistically significant difference between these specific groups. In contrast, all other pairwise comparisons show significant differences (p-adj < 0.05), indicating that the pH levels in these groups differ in a statistically reliable manner.

Also the studied bacteriocins have an optimal pH of around 6, while maintaining inhibitory activity at a pH of 2, highlighting its ability to adapt to acidic conditions. This characteristic is particularly noteworthy as it allows the bacteriocin to retain its effectiveness in various environments, including those typical of certain industrial applications. Indeed, previous studies have shown that many bacteriocins maintain activity across a pH range of 2 to 8, although the intensity of their effectiveness may vary depending on the specific bacteriocin and the bacterial strain targeted (Cotter et *al*., 2005). For example, nisin, a widely studied class IIa bacteriocin, remains active against various foodborne pathogens within a pH range of 2 to 7. This ability to withstand acidic conditions while retaining maximum efficacy at neutral pH represents a major advantage for its use in food preservation (Delves-Broughton et *al*., 1996). The relevance

Table 2. Antimicrobial activity under different treatments

Souches	Traitement		
	Untreated	Treated with Proteinase K	Treated with Trypsin
F2-1	+	-	-
H3-1	+	-	-
H2-2	+	-	-
H2-1	+	-	-
H1-1	+	-	-

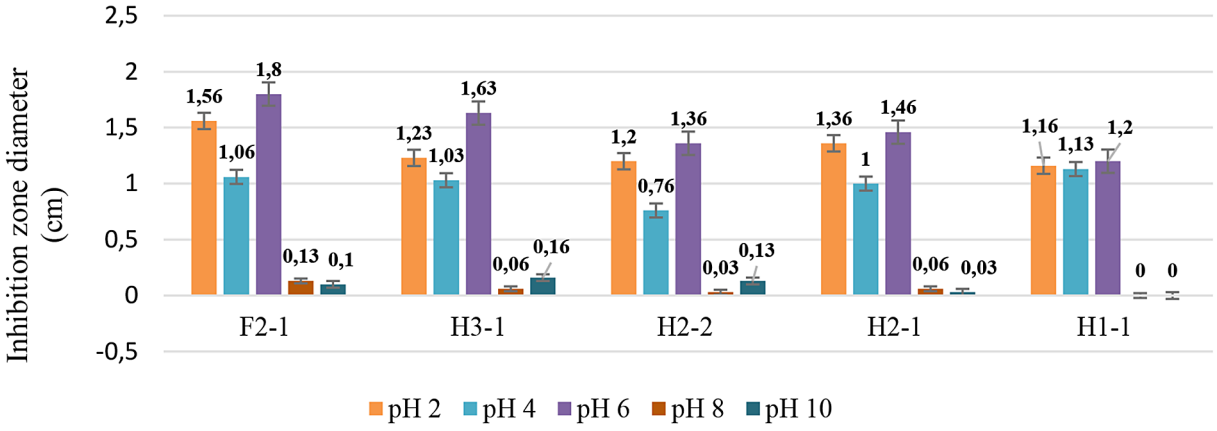


Figure 4. Effect of pH on bacteriocins activity

Figure 5. A) Inhibition zone produced by strain F2-1 against *Listeria innocua*
B) Inhibition zone produced by strain H3-1 against *Listeria innocua*

of such properties for the food industry is significant. Bacteriocins, particularly nisin, are used as natural preservatives in a range of food products, including cheeses, meats, and beverages, where acidic conditions are common. Their ability to inhibit pathogen growth while remaining stable in environments with variable pH helps extend the shelf life of food products without the need for potentially controversial chemical additives (Gänzle and Gobbetti, 2012).

Effect of temperature

At relatively high temperatures, the tested bacteriocins exhibited a certain degree of resistance, although their activity decreased after exposure to 80 and 121 °C for 20 minutes. In contrast, both bacteriocins maintained their stability at low temperatures, particularly at 4 °C (Figure 6). An example of the results is shown in Figure 7. The results of the statistical analysis are summarized in the Table 5.

Table 3. Descriptive statistics of bacteriocin activity at different pH values

Parameter	pH 2	pH4	pH6	pH8	pH10
Min	1.7	0.77	1.20	0.00	0.00
Mean	1.31	1.00	1.49	0.06	0.09
Median	1.23	1.03	1.47	0.07	0.10
Mode	/	/	/	0.06666667	/
Standard deviation	0.16397832	0.13944334	0.23261795	0.04944132	0.06912147
Max	1.57	1.13	1.80	0.13	0.17

Table 4. Tukey post-hoc results

Comparaison	Adjusted P-value	Conclusion
pH 2 vs pH 10	0.0000	Significant difference
pH 2 vs pH 4	0.0260	Significant difference
pH 2 vs pH 6	0.2965	No significant difference
pH 2 vs pH 8	0.0000	Significant difference
pH 10 vs pH 4	0.0000	Significant difference
pH 10 vs pH 6	0.0000	Significant difference
pH 10 vs pH 8	0.9984	No significant difference
pH 4 vs pH 6	0.0003	Significant difference
pH 4 vs pH 8	0.0000	Significant difference
pH 4 vs pH 8	0.0000	Significant difference

Application of ANOVA resulted in an F-ratio of 44.8201 and a p-value below 0.0001, confirming statistically significant differences among temperature groups. The Table 6 presents the results of the Tukey post-hoc test.

Tukey's post hoc test revealed statistically significant differences between several temperature pairs. For instance, comparisons such as 120 °C vs 30 °C, 120 °C vs 45 °C, and 120 °C vs 4 °C yielded very low p-values ($p < 0.05$), indicating that the differences between these groups are statistically significant. Similarly, significant differences were also observed between 30 °C vs 4 °C, 30 °C vs 80 °C, 45 °C vs 4 °C, and 45 °C vs 80 °C.

In contrast, no significant differences were found between 120 °C vs 80 °C, 30 °C vs 45 °C, and 4 °C vs 80 °C, as indicated by p-values greater than 0.05. This suggests that the response levels measured at these temperatures do not differ in a statistically meaningful way.

These results highlight its potential application in food safety, offering flexibility in various food storage and processing conditions.

Recent studies have confirmed that certain bacteriocins, such as nisin, remain active even at high temperatures, often exceeding 80 °C, although their activity is generally reduced at extreme temperatures. For example, a study by Zhang et *al.* (2021)

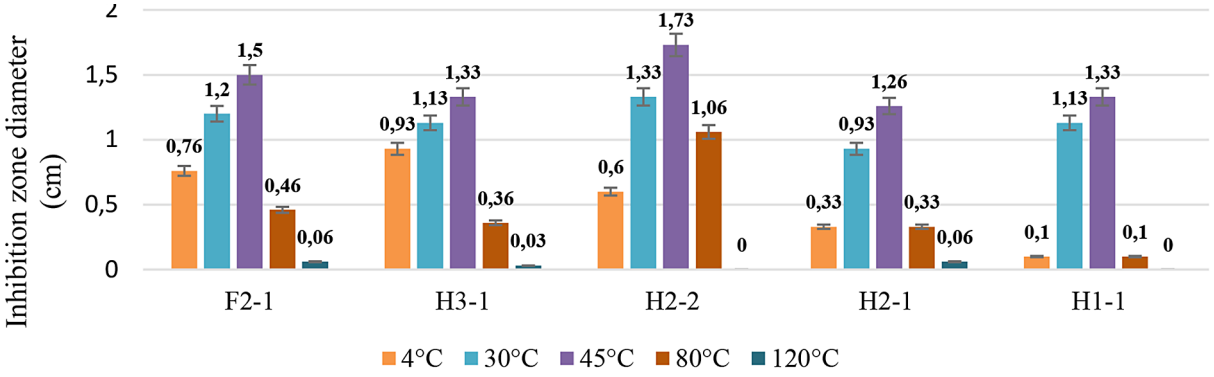


Figure 6. Effect of temperauture on bacteriocins activity

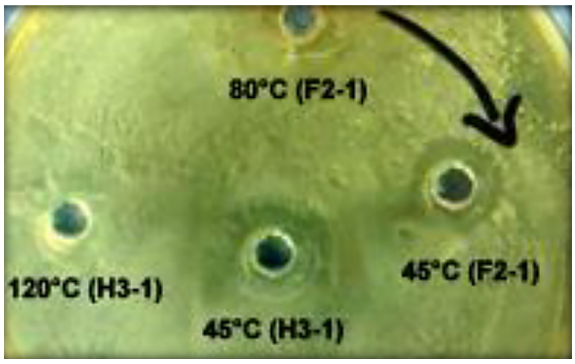


Figure 7. Effect of temperature on bacteriocins activity

observed that nisin retains its antimicrobial activity after exposure to 90 °C, although its efficacy is attenuated at very high temperatures. This characteristic is particularly relevant for thermal processing of foods, where the bacteriocin can help inhibit the growth of heat-resistant pathogens such as Listeria monocytogenes (García-López et al., 2023).

Moreover, the bacteriocin's ability to maintain antimicrobial activity at 4 °C is of great importance for the preservation of refrigerated products. At this temperature, where pathogen proliferation is slowed, bacteriocins like nisin have been shown to be effective against Listeria monocytogenes and other pathogens (Liu et *al*., 2024). These results confirm that the use of bacteriocins could provide a viable solution to limit microbial risks in refrigerated products, where contaminants can still pose a food safety concern.

Effect of anionic, denaturing, and surfactant agents on antimicrobial activity

The different anionic agents and surfactants tested exhibited variable antimicrobial activity in the tested strains (Figure 8), with a representative example shown in Figure 9. The results of the statistical analysis are summarized in Tables 7 and 8.

Tukey's post hoc analysis indicated statistically significant differences between several treatment pairs. Notably, significant differences ($p < 0.05$) were observed between EDTA and H_2O_2, H_2O_2 and NaCl, H_2O_2 and SDS, H_2O_2 and Triton X-100, H_2O_2 and urea, as well as Triton X-100 and Tween 80.

Conversely, no significant differences were detected among the remaining pairs, including EDTA vs NaCl, SDS, Triton X-100, Tween 80, and urea; H_2O_2 vs Tween 80; NaCl vs SDS, Triton X-100, Tween 80, and urea; SDS vs Triton X-100, Tween 80, and urea; Triton X-100 vs urea; and Tween 80

Table 5. Descriptive statistics of bacteriocin activity at different temperatures

Parameter	4 °C	30 °C	45 °C	80 °C	120 °C
Min	0.10	0.93	1.27	0.10	0.00
Mean	0.55	1.15	1.43	0.29	0.03
Median	0.60	1.13	1.33	0.33	0.03
Mode	/	/	1.33333333	/	0.06666667
Standard deviation	0.33383296	0.14832397	0.18856181	0.15018507	0.03333333
Max	0.93	1.33	1.73	0.47	0.07

Table 6. Tukey post-hoc results

Comparaison	Adjusted P-value	Conclusion
120 °C vs 30 °C	0.0000	Significant difference
120 °C vs 45 °C	0.0000	Significant difference
120 °C vs 4 °C	0.0042	Significant difference
120 °C vs 80 °C	0.2836	No Significant difference
30 °C vs 45 °C	0.2006	No Significant difference
30 °C vs 4 °C	0.0008	Significant difference
30 °C vs 80 °C	0.0000	significant difference
45 °C vs 80 °C	0.0000	Significant difference
45 °C vs 4 °C	0.0000	Significant difference
4 °C vs 80 °C	0.2609	No Significant difference

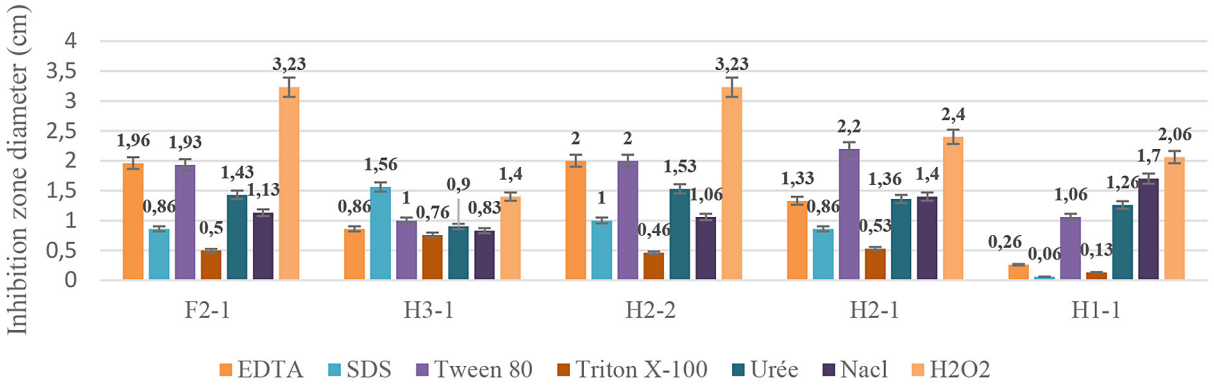


Figure 8. Effect of anionic, denaturing, and surfactant agents on bacteriocins activity

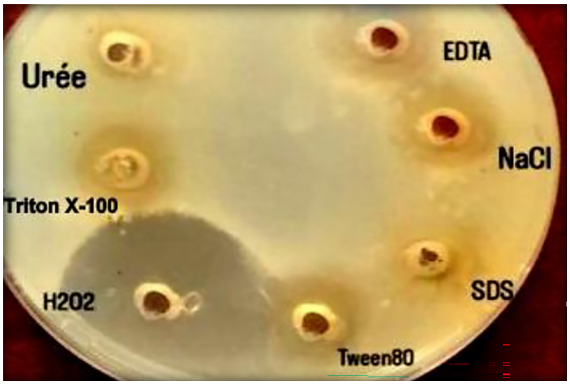


Figure 9. Effect of anionic, denaturing, andsurfactant agents on bacteriocins activity of F2-1 strain

vs urea, indicating comparable effects among these treatments on the measured parameter.

The addition of hydrogen peroxide (H_2O_2) to the supernatant containing bacteriocins resulted in a marked increase in the inhibition zone against Listeria innocua, likely due to membrane disruption that facilitates bacteriocin entry, thereby enhancing antimicrobial efficacy (Zhang et *al*., 2023).

The increased inhibition halos observed following treatment of the bacteriocin-containing supernatant with EDTA and Tween 80 can similarly be attributed to their ability to compromise membrane integrity in *Listeria innocua* cells.

EDTA, as a chelating agent for divalent cations, destabilizes the cell membrane by disrupting essential ionic interactions, thereby rendering the bacteria more susceptible to bacteriocin action (Pranoto et *al*., 2005).

Tween 80, due to its surfactant properties, alters membrane permeability, facilitating the insertion or action of bacteriocins, which likely explains the observed zones of clearance. These findings are consistent with those reported by Settanni et *al*. (2008).

In the case of strain F2-1, only SDS appeared to slightly enhance antimicrobial activity. This effect is primarily attributed to its denaturing action, which at low concentrations (0.1–1%) facilitates bacteriocin activity, although higher concentrations may compromise their stability (Parvez et *al*., 2006).

Classification of bacteriocins

The bacteriocins produced by our strains, which exhibit significant inhibitory activity against *Listeria innocua* but not against *Pseudomonas aeruginosa* or *Escherichia coli*, can be classified as class II. These bacteriocins are low molecular weight peptides known for their strong specificity against Listeria spp. and their remarkable stability under extreme conditions, such as acidic pH (pH 2) and high temperatures (80 °C).

Our findings align with those of Garcia-Lopez et *al*. (2023), who demonstrated the efficacy of *Lb. paraplatarum* BPF2 bacteriocins against *Listeria monocytogenes*. Similarly, Hartmann et *al*. (2011) reported the absence of inhibitory activity of *Lb. plantarum* DSM1055 against *Listeria monocytogenes* under comparable conditions. Additionally, class II bacteriocins, such as pediocin PA-1/AcH, are well-recognized for their specificity and stability, which are crucial for their application in food safety (Drider et *al*., 2006; Ennahar et *al*., 2000). These findings strongly support the classification of our strains within this category, highlighting their potential as targeted antimicrobial agents against *Listeria innocua*.

Further studies underline the practical advantages of class II bacteriocins. Motta and Brandelli (2008) emphasized their resistance to proteolytic enzymes and their sustained antimicrobial activity, which makes them highly valuable for food

Table 7. Descriptive statistics of bacteriocin activity at different anionic, denaturing, and surfactant agents

Parameter	EDTA	SDS	Tween 80	Triton X-100	Urée	Nacl	H_2O_2
Min	0.27	0.07	1.00	0.13	0.90	0.83	1.40
Mean	1.35	0.87	1.64	0.48	1.23	1.21	2.47
Median	1.67	0.87	1.93	0.50	1.37	1.07	2.40
Mode	/	0.86666667	/	/	/	/	3.23333333
Standard deviation	0.75997076	0.5356201	0.56292885	0.22681368	0.29533409	0.34270168	0.78704793
Max	2.00	1.57	2.20	0.77	1.53	1.70	3.23

Table 8. Tukey post-hoc results

Comparaison	Adjusted P-value	Conclusion
EDTA vs H_2O_2	0.0422	Significant difference
EDTA vs NaCl	0.9994	No significant difference
EDTA vs SDS	0.7972	No significant difference
EDTA vs Triton X-100	0.1812	No significant difference
EDTA vs Tween 80	0.9788	No significant difference
EDTA vs Urea	0.9998	No significant difference
H_2O_2 vs NaCl	0.0153	Significant difference
H_2O_2 vs SDS	0.0013	Significant difference
H_2O_2 vs Triton X-100	0.0001	Significant difference
H_2O_2 vs Tween 80	0.2312	No significant difference
H_2O_2 vs Urea	0.0185	Significant difference
NaCl vs SDS	0.9559	No significant difference
NaCl vs Triton X-100	0.3693	No significant difference
NaCl vs Tween 80	0.8622	No significant difference
NaCl vs Urea	1.0000	No significant difference
SDS vs Triton X-100	0.9074	No significant difference
SDS vs Tween 80	0.3091	No significant difference
SDS vs Urea	0.9372	No significant difference
Triton X-100 vs Tween 80	0.0308	Significant difference
Triton X-100 vs Urea	0.3285	No significant difference
Tween 80 vs Urea	0.8935	No significant difference

preservation. Todorov and Dicks (2005) also demonstrated their robustness and efficacy under diverse environmental conditions, reinforcing their utility as biopreservatives.

Recent research has expanded the understanding of the antimicrobial potential of *Lactobacillus* strains against foodborne pathogens. In particular, bacteriocins from *Lactobacillus* are increasingly recognized for their stability under food processing conditions, enhancing their applicati-on in food preservation. Moreover, the resilience of class II bacteriocins to environmental stresses underscores their importance for industrial and clinical applications (Cheng et *al*., 2023). Their ability to selectively target pathogens like Listeria while maintaining efficacy under challenging conditions positions them as ideal candidates for improving food safety.

Identification of lactic acid bacteria strains using the API 50CH gallery

The results enabled us to select two strains exhibiting strong antimicrobial activity among the five strains tested: F2-1 and H3-1.

According to the phenotypic characteristics of H3-1 and F2-1 strains (Figure 1) – to the metabolic profile obtained using the API 50 CHL identification system, the strains were assigned as:

- F2-1: *Lactobacillus plantarum,*
- H3-1: *Lactobacillus pentosus.*

CONCLUSIONS

The present study demonstrates that lactic acid bacteria strains isolated from *Hamoum* and figs soaked in olive oil exhibit notable antimicrobial potential, particularly against *Listeria innocua*. The results indicate that the molecules responsible for this activity are bacteriocins, which are sensitive to proteolytic enzymes, confirming their proteinaceous nature. These bacteriocins also exhibit remarkable stability under extreme conditions, including low pH (pH 2) and elevated or low temperatures (4 °C and 80 °C), making them suitable for industrial applications. Among the tested anionic, denaturing, and surfactant agents, hydrogen peroxide (H_2O_2) significantly enhanced the antimicrobial activity, suggesting its role in facilitating bacteriocin action. The antimicrobial properties of these bacteriocins, such as their stability and specificity against *Listeria*, classify them as class II bacteriocins, making them promising candidates for combating foodborne pathogens.

The identification of the strains as *Lactobacillus plantarum* and *Lactobacillus pentosus* further supports their potential use as biopreservative agents in the food industry.

The application of these strains offers a natural and effective alternative to conventional preservation methods, thereby reducing reliance on chemical preservatives. Their incorporation into biopreservation systems could also help extend the shelf life of food products while ensuring microbiological safety.

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