Malaysian Journal of Microbiology, Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Preparation of anti-staphylococcal packaging material using antimicrobial metabolite from *Lactiplantibacillus plantarum* strain N1 isolated from fermented sausage from Egypt

Noha Adel Ali[1]*, Einas Hamed El-Shatoury[1], Asrar Yaseen Mohamed[2] and Ali Mohamed Saeed[1]

[1]Department of Microbiology, Faculty of Science, Ain Shams University, Cairo 11566, Egypt.
[2]Food Technology Research Institute, Agricultural Research Center, Egypt.
Email: nohaadel0103841@sci.asu.edu.eg

Received 20 April 2023; Received in revised form 5 November 2023; Accepted 11 January 2024

ABSTRACT

Aims: This study was aimed to produce biodegradable cellulose acetate films impregnated with bacteriocin-like inhibitory substances to be used in food packaging.
Methodology and results: Bacterial isolates were isolated from different sources and tested for their antimicrobial activity by agar well diffusion assay. The isolate that showed the highest antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 51659, *Pseudomonas aeruginosa* MG847103 and *Streptococcus mutans* ATCC 25175 was identified as *Lactiplantibacillus plantarum* strain N1 (OM019104) based on 16S rRNA based method of identification. The extracted bacteriocin-like inhibitory substances were partially purified with acetone precipitation and SP-Sephroase cation exchange chromatography. There was no change in the antimicrobial activity after treatment with the catalase enzyme, but there was a total loss in the activity after treatment with proteolytic enzymes. The obtained bacteriocin-like inhibitory substances showed pH stability over a wide range of pH values and thermal stability as it recovered 95% of its antimicrobial activity even after autoclaving for 15 min. Different concentrations of cellulose acetate (3 to 12%) were tested to develop the active antimicrobial films. The most flexible concentrations for food packaging were prepared and impregnated with partially purified bacteriocin-like inhibitory substances. The prepared films showed promising antimicrobial activity against *S. aureus.*
Conclusion, significance and impact of study: This study highlights the usage of active packaging in food preservation. Cellulose acetate films activated with the partially purified bacteriocin-like inhibitory substances have anti-staphylococcal activity, which can potentially be used in food packaging to prolong the shelf-life of perishable foods.

Keywords: Antimicrobial food packaging, bacteriocin, cellulose acetate, lactic acid bacteria

INTRODUCTION

Food spoilage by pathogens or their toxins significantly impacts food quality and shelf-life and may cause foodborne outbreaks (Villalobos-Delgado *et al*., 2019). There are many methods for food preservation and prevention of food spoilage, such as freezing, drying, refrigeration, fermentation and adding chemical additives (Amit *et al*., 2017). Recently, a new concept appeared in the food industry called active packaging (Amin *et al*., 2022). It provides interaction between food and packaging materials to maintain a microenvironment. One of the most promising types of active packaging is antimicrobial packaging (Huang *et al.*, 2019), the incorporation of antimicrobial substances into packaging materials to inhibit the growth of microorganisms on the surface of solid or semi-solid food. Various natural and synthetic substances are used in antimicrobial packaging, such as benzoate, metallic ions, sorbates, organic acids and bacteriocins (Santiago-Silva *et al*., 2009; Verma *et al*., 2022). In recent years, the use of biodegradable films as carriers of antimicrobial substances such as bacteriocins to produce active packaging is a promising concept in the food industry (Bhattacharya *et al*., 2022).

Bacteriocin is a heterogeneous group of ribosomally synthesized antimicrobial proteins produced by different groups of bacteria (Rea *et al*., 2011). Many lactic acid bacteria (LAB) can produce bacteriocins (Fernandes and Jobby, 2022). Bacteriocins produced by LAB are particularly interesting due to the long history of their safe use and the generally regarded as safe (GRAS) statute (Alvarez-Sieiro *et al.*, 2016). In recent years, bacteriocin has attracted huge interest due to the increasing demand for food free of chemical additives and the increase in antibiotic-resistant bacteria due to the excessive use of antibiotics in the food industry (O'Connor *et al*., 2020).

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

Also, bacteriocin are proteins in nature, so they do not alter the intestinal microbiota as they are degraded by intestinal enzymes such as trypsin and pepsin (Contessa *et al.*, 2021).

The incorporation of antimicrobial substances into food packaging is an exciting way to preserve food products and increase their shelf-life due to the combination of the preservative effect of antimicrobial substances and the protective function of packaging (Scannell *et al*., 2000; Manzoor *et al*., 2023). Several studies over the years described active packaging in the food industry to increase the shelf-life of food products by incorporating bacteriocin as natural antimicrobial substances into different packaging materials for food preservation (Salvucci *et al*., 2019; Contessa *et al*., 2021; Giello *et al*., 2023). Polysynthetic polymers such as polyethylene, polypropylene and polyvinyl chloride are widely used in the food packaging industry (Iseppi *et al*., 2008; La Storia *et al*., 2008; Barbosa *et al*., 2013). Several studies tested films incorporated with bacteriocin as packaging material for the preservation of different food products such as minimally processed fruits (Zhang *et al*., 2017), fish (Woraprayote *et al*., 2018) and dairy products such as cheese (Contessa *et al*., 2021). Polysynthetic polymers are non-biodegradable and their accumulation in the environment causes huge impacts and environmental problems. In the last decade, many studies have used alternatives to polysynthetic polymers like bio-based materials (Yang *et al*., 2020) and green eco-friendly polymers like cellulose acetate (Barbosa *et al*., 2013; Marrez *et al*., 2019). The integration of bacteriocin into packaging material can be done using several methods, such as direct blending into the packaging material matrix (Sun *et al.*, 2019), coating on packaging material (Imran *et al*., 2010), or chemical immobilization (Marvdashti *et al*., 2019). Cellulose acetate is an eco-friendly polymer derived from cellulose. This polymer has many characteristics that make it a good choice for food packaging. Some of these characteristics are insolubility in water, the capability to form films at room temperature, non-toxicity and the minimum affinity to water vapor (Gouvêa *et al*., 2015). Recently, many studies have used cellulose acetate as packaging material to develop active food packaging (Assis *et al*., 2021; Zhang *et al*., 2022). This study used cellulose acetate as a biodegradable material to develop food packaging films by coating developed films with partially purified BLIS extracted from *L. plantarum* strain N1.

This study aimed to isolate LAB, extract, and purify bacteriocin-like inhibitory substances (BLIS) from LAB isolates that showed the highest antimicrobial activity against tested pathogens. Moreover, studying the thermal stability and pH stability of the produced BLIS. Finally, develop eco-friendly, biodegradable cellulose acetate films impregnated with the produced partially purified BLIS to be used in food packaging.

MATERIALS AND METHODS

Bacterial strains and materials

Indicator pathogens include two Gram-positive bacteria and two Gram-negative bacteria. *Staphylococcus aureus* ATCC 29213 *S. mutans* ATCC 25175 and *E. coli* ATCC 51659 were obtained from the American Type Culture collection and *P. aeruginosa* strain E1 (MG847103) was kindly supplied from the Microbiology Department, Faculty of Science. De Man, Rogosa and Sharpe media (MRS Broth with Tween 80, Biolife Italiana, Italy, product number 401729). Cellulose acetate (40% wt acetyl content, Avg. Mn: 37000, Aldrich) and acetone as a local product was obtained from El Naser Pharmaceutical Chemical Company, Egypt. Trypsin (Sigma, USA, product number T2600000), proteinase K (Sigma, USA, product number P2308) and catalase (Sigma, USA, product number C9322) were purchased from Sigma (all 1 mg/mL).

Samples collection

Three different samples of the following sources, raw cow milk, natural yogurt and traditionally fermented sausage, were collected from the local market in Egypt in sterile containers and transported to the laboratory under appropriate conditions. Cow milk was delivered from the cowshed, while yogurt and sausage were purchased from a small supermarket in Cairo, Egypt. In this study, different food product samples are collected to isolate lactic acid bacteria.

Isolation and characterization of lactic acid bacteria

The ten-fold dilution was carried out in distilled sterile water for each sample, then one mL of each dilution was plated in triplicates in de Man, Rogosa and Sharpe media (MRS broth, Biolife Italiana, Italy) and plates were incubated at 37 °C for 24 and 48 h under anaerobic conditions. After incubation, colonies with different morphology, shape, color, and size were selected, streaked on MRS agar plates, and incubated under the same conditions. Isolates were selected from plates and stored in 30% glycerol at -70 °C for further investigations (Bostan *et al*., 2017; Islam *et al*., 2021).

Isolates were characterized using Gram stain, catalase test, growth at different concentrations of NaCl (3%, 6.5% and 10%) and tolerance to acidity. Gram-positive and catalase-negative isolates, able to grow in different concentrations of NaCl and tolerate acidity, were supposed to be lactic acid bacteria (LAB) (Papamanoli *et al.*, 2003).

Determination of antimicrobial activity of selected isolates

Selected LAB were inoculated in fresh MRS media for 16 h at 37 °C. After incubation, cells were discarded by centrifugation at 8000 rpm for 3 min to obtain the cell-free

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

supernatants (CFS). The cell-free supernatant was adjusted to pH 6-6.5 using 1 mol/L NaOH to exclude the effect of organic acids and filtered through a 0.22 µm filter (Millipore, United States). The antimicrobial activity of CFS was determined in triplicates using an agar well diffusion assay with some modifications in the diameter of wells. Cell-free supernatant (100 µL) was placed in 6 mm wells on plates inoculated with indicator pathogens *S. aureus* ATCC 29213, *E. coli* ATCC 51659, *S. mutans* ATCC 25175 and *P. aeruginosa* strain E1 (MG847103). Diffusion for 2 h at 4 °C was allowed and then the plates were incubated at 37 °C for 24 h. For control, wells were inoculated with distilled sterile water. After incubation, the inhibition zones were measured to determine the antimicrobial activity of CFS (Zhang *et al*., 2018). The most potent isolate was selected based on the antimicrobial activity of CFS. The cell-free supernatant recovered from the most potent isolate was further characterized including effect of enzymes, effect of pH and thermal stability.

Effect of enzymes and pH on the activity of BLIS

To confirm the proteinaceous nature of the extracted CFS and exclude the probability that the antimicrobial activity might be due to the presence of hydrogen peroxide that might be produced by bacterial isolate and found in the CFS. One mL of CFS was treated with trypsin, proteinase K, and catalase (all 1 mg/mL, all Sigma) incubated at 37 °C. After 2 h of incubation, the mixture was heated at 95 °C for 10 min to inactivate the enzymes. The antimicrobial activity of the treated CFS was tested in triplicates against *S. aureus* (Sidhu and Nehra, 2021). Untreated samples were used as a control.

The pH sensitivity of BLIS was tested at various pH values ranging from 2.0 to 12.0. Aliquots of 1 mL from CFS were adjusted to different pH using either HCL or NaOH and incubated for 2 h at 37 °C. After incubation, the treated aliquots were readjusted to 6-6.5. Both treated and untreated samples (control) were tested for triplicate antimicrobial activity (Zhang *et al*., 2018; Sultan and Hatice, 2023).

Thermal stability of BLIS

Aliquots of 1 mL from CFS were treated at different temperatures for different periods to determine the thermal stability of BLIS (65 °C and 90 °C for 20, 30 and 60 min), (100 °C for 10, 20 and 30 min) and 121 °C for 20 min (autoclaving). The antimicrobial activity of heat-treated samples and untreated control samples (not exposed to heat treatment) were measured in triplicates using agar well diffusion assay (Rossi *et al*., 2021; Sadeghi *et al*., 2023).

Purification of BLIS

The obtained supernatants were partially purified using the acetone precipitation method (Yap *et al*., 2022). Supernatants were treated with four volumes of cold acetone, stored at -20 °C overnight and then centrifuged to recover the peptide fractions in the pellet (Saavedra and Sesma, 2011). The pellet was suspended in distilled and sterile water. Then, lyophilized to decrease the working volume.

The lyophilized partially purified BLIS was re-suspended in 50 m/mol sodium phosphate buffer (pH 6.0) at a concentration of 5 mg/mL and then subjected to fast protein liquid chromatography (FPLC)-cation exchange chromatography on an SP-Sephroase F column (5 mL) (GE Healthcare) using an AKTA Avant (GE Healthcare) at central laboratories network, National Research Center, Cairo, Egypt. The absorbed material was eluted with a linear gradient of 0-1 mol/ L NaCl at a 2 mL/min flow rate. A UV detector at 220 nm was used to monitor the elution process. The antimicrobial activity of the obtained fractions was determined in triplicates against indicator pathogens using a well diffusion assay. The active protein fractions were lyophilized and stored at -20 °C (Tumbarski *et al*., 2018).

Molecular identification of the most potent isolate

The DNA of the most potent bacterial isolate was extracted using DNA extraction kits (Thermo; Fisher Scientific; USA). The bacterial universal primers 27F (5′-AGA GTT TGATCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) were used for PCR amplification of the selected isolate's 16S rRNA gene. The PCR reaction was performed in the genius model FGENO2TD thermal cycler (Techne, England) and Maxima Hot Start PCR Master Mix (Thermo K1051) was used according to the manufacturer's protocols. The PCR reaction was carried out as follows: initial denaturation at 95 °C for 5 min, then 35 cycles of 1 min at 95 °C, 1 min at 54 °C and 1 min at 72 °C, and finally 10 min at 72 °C for amplification of genes. To visualize the amplified genes, 1% agarose gel with a size marker was used. This step was carried out to determine the size and purity of the amplified genes. Amplified bands were cleaned using GeneJET™ PCR Purification Kit (Thermo K0701).

Sequencing was performed by using an ABI 3730xl DNA Sequencer in GATC Company (Konstanz, Germany). The obtained sequence was identified by using the blast search program National Center for Biotechnology Information (NCBI) (Altschul *et al*., 1997). Alignment of the sequence and phylogenetic tree was constructed using the neighbor-joining method using MEGA V6 software (Tamura *et al*., 2013). The obtained sequence was submitted to NCBI to obtain an accession number.

Development of biodegradable, antimicrobial packaging films by using BLIS

Cellulose acetate films were prepared by using the casting method according to Alvarez-Sieiro *et al*. (2016). Different cellulose acetate concentrations (3 to 12% v/w) were prepared to determine the best concentration to be used in film production. The thickness of the films was

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

measured using a micrometer. For further experiments, the produced films were sterilized for 2 min on each side by using UV (Barbosa *et al*., 2013).

Cellulose acetate films were sliced into circles (10 mm diameter) and soaked into 10 mL of partially purified BLIS (1000 AU/mL) solution for 2 h at 4 °C under static conditions. After soaking, the disks were washed three times with deionized water to remove the unabsorbed BLIS and then dried at 37 °C.

The antimicrobial activity of the films

The antimicrobial activity of the produced films was tested in triplicates against the indicator pathogen *S. aureus* ATCC 29213 (The most affected pathogen with the antimicrobial activity of the obtained BLIS) using disk diffusion assay (Barbosa *et al*., 2013). Approximately 10^5 CFU/mL of the indicator pathogen was inoculated on the surface of solid media, then the sterile 10 mm diameter disks were transferred to the surface of the plates, diffused for 2 h at 4 °C and incubated at 37 °C for 24 h. After incubation, the growth inhibition zones were measured (Zhang *et al.*, 2017). A control treatment without BLIS was also included.

Statistical analysis

All statistical analysis in this study was carried out using analysis of variance (ANOVA, SPSS software version 18) followed by the Duncan test at 0.05 level. $^{a\text{-}e}$ means with different superscripts in the same column are considered statistically different (P≤0.05). All data were calculated from at least 3 replicates and the standard error for each datum was plotted on the graph.

RESULTS

Isolation and characterization of lactic acid bacteria

Sixteen isolates were recovered from collected samples; these isolates were differentiated based on their morphological characteristics. All sixteen isolates were Gram-positive and catalase-negative. Ten of sixteen isolates were able to grow in different concentrations of NaCl (3 %, 6.5% and 10 %) and tolerate different levels of acidity, as shown in Table 1. Out of sixteen isolates, ten isolates were supposed to be lactic acid bacteria.

Determination of antimicrobial activity of selected isolates

The obtained cell-free supernatant (CFS) from the ten selected isolates were tested in triplicates for antimicrobial activity against indicator pathogens (*S. aureus* ATCC 29213, *P. aeruginosa* strain E1 MG847103, *E. coli* ATCC 51659 and *S. mutans* ATCC 25175*)* using well diffusion assay. The result showed that the selected isolates had antimicrobial activity against indicator pathogens with variable levels. The inhibition zone of CFS against *S. aureus* ranged from 14.6 mm to 15 mm, *P. aeruginosa* ranged from 13.8 mm to 14.1 mm, *E. coli* ranged from 12.3 mm to 12.5 mm and *S. mutans* ranged from 13.1 mm to 13.5 mm. Out of ten selected isolates, the isolate that showed the highest antimicrobial activity against indicator pathogens *S. aureus* ATCC 29213 (15 ± 0.04 mm), *E. coli* ATCC 51659 (12.5 ± 0.1 mm), *P. aeruginosa* strain E1 MG847103 (14.1 ± 0.2 mm) and *S. mutans* ATCC 25175 (13.5 ± 0.1 mm), was the isolate N1 (obtained from sausage samples), as shown in (Figure 1) so that it was used in further experiments.

Effect of enzymes and pH on the activity of BLIS

Treatment of the CFS produced by strain N1 with proteolytic enzymes resulted in a reduction of the antimicrobial activity, which indicates the proteinaceous nature of CFS.

The effect of enzymes on CFS was studied using three enzymes (trypsin, proteinase K and catalase). There was no change in the antimicrobial activity of CFS after treatment with catalase enzyme (20 ± 0.2 mm, 100% activity), but there was a total loss in antimicrobial activity after treatment with proteolytic enzymes (trypsin and proteinase K), as shown in Table 2.

The effect of different ranges of pH on the activity of BLIS is shown in (Figure 2a). BLIS was stable over a wide

Table 1: Characterization of the selected isolates to differentiate between lactic acid bacteria and non-lactic acid bacteria.

Isolate Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth in NaCl																
3%	+	+	+	+	+	++	+	++	+	+	-	+	+	-	-	-
6.5%	+	-	++	-	-	+	+	+	++	+	-	-	-	-	-	-
10%	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Growth in different pH levels																
pH 5.3	+	-	+	++	+	+	+	++	++	+	-	-	-	-	-	-
pH 4.25	+	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-
pH 3	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

- = No growth; + = Moderate growth; ++ = Maximum growth.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

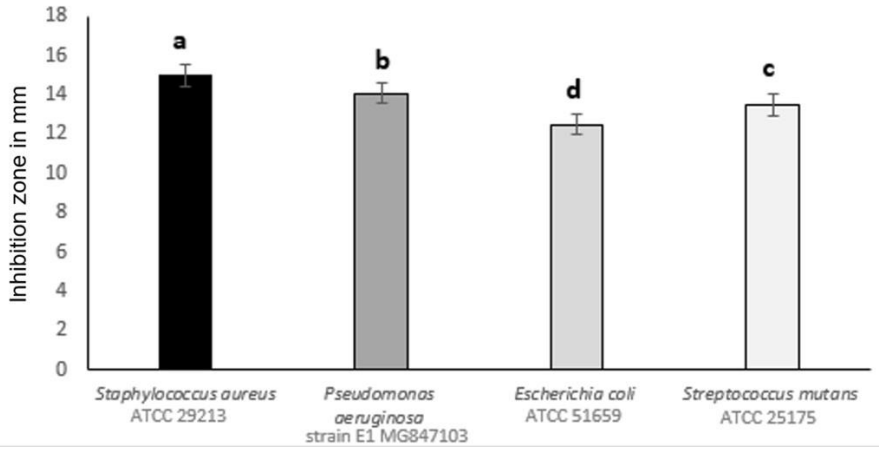

Figure 1: Antimicrobial activity of cell free supernatant produced from the most potent isolate strain N1 against indicator pathogens. Data are shown as the mean ± SE of triplicate measurements from independent experiments. Statistical significance was assessed using a one-way ANOVA (analysis of variance, SPSS software v.18) test and the means were compared with Duncan's test at 0.05 level. [a-d] Accordingly, mean values with different small letters are considered statistically different (P≤0.05).

Table 2: The effect of temperature and enzymes on the activity of the produced cell free supernatant from the most potent isolate strain N1

Treatment	Bacteriocin activity (inhibition zone in mm)	Residual inhibitory activity (%)
Temperature		
65 °C for 20 min	18.3 ± 0.1[ab]	91.5
65 °C for 30 min	18 ± 0.09[b]	90
65 °C for 60 min	17 ± 0.1[c]	85
90 °C for 20 min	19 ± 0.09[a]	95
90 °C for 30 min	18.6 ± 0.2 [ab]	93
90 °C for 60 min	18.3 ± 0.1 [ab]	91.5
100 °C for 10 min	19 ± 0.4 [a]	95
100 °C for 20 min	18 ± 0.09 [b]	90
100 °C for 30 min	16.3 ± 0.2 [c]	81.5
121 °C for 20 min (Autoclaving)	19 ± 0.09 [a]	95
Enzymes		
Catalase	20 ± 0.2	100
Proteinase K	0	0
Trypsin	0	0

The results are the inhibition zones expressed in mm as the mean of three replicates, ± standard error. Statistical significance was assessed using a one-way ANOVA (analysis of variance, SPSS software v.18) test and the means were compared with Duncan's test at 0.05 level. [a-c] Accordingly, mean values with different small letters are considered statistically different (P≤0.05).

range of pH 2.0 to 12.0. BLIS retained 78.9% of its activity when kept at pH 12.0 for 2 h and retained 73.69 of its activity when kept at pH 2.0 for 2 h. The optimal pH for activity was pH 6 (20 ± 0.09 mm, 100% activity) and the antimicrobial activity was expressed over a wide range of pH values. There was no complete loss of activity with any pH treatment.

Thermal stability of BLIS

BLIS showed high levels of thermal stability in different temperature values. The BLIS maintained 90% of its activity at 90 °C for 60 min and at 100 °C for 20 min. And maintained 95% of its antimicrobial activity even after autoclaving (121 °C for 20 min). The BLIS showed a significantly slight decrease (P<0.05) in the antimicrobial activity by increasing the heat treatment time with all tested temperature degrees (Figure 2b and Table 2).

Purification of BLIS

The crude bacteriocin extract (cell-free supernatant) from the selected isolate was partially purified using the acetone precipitation method. The precipitate was dissolved in distilled water and tested for antimicrobial activity. The result showed that the precipitate had higher

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

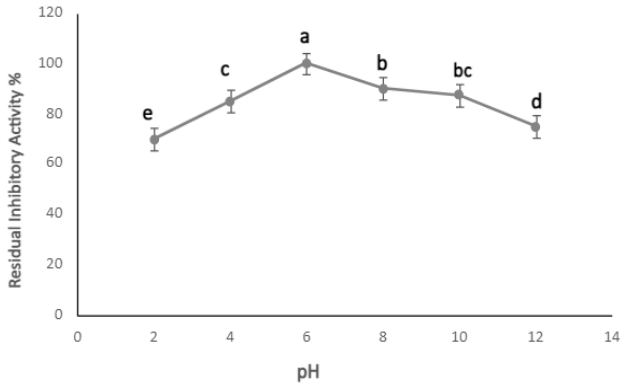

Figure 2a: The effect of different pH values (2.0 to 12.0) on the activity of cell free supernatant produced from the most potent isolate (strain N1). Data are shown as the mean ± SE of triplicate measurements from independent experiments. Statistical significance was assessed using a one-way ANOVA (analysis of variance, SPSS software v.18) test and the means were compared with Duncan's test at 0.05 level. [a-e] Accordingly, mean values with different small letters are considered statistically different (P≤0.05).

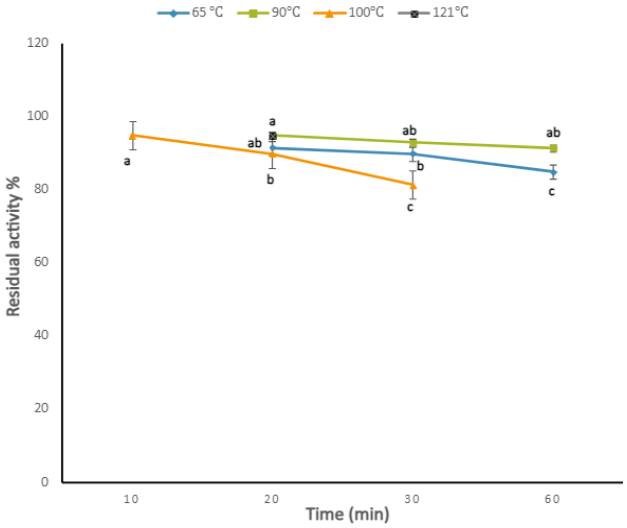

Figure 2b: Thermo-stability of cell free supernatant produced from the most potent isolate strain N1 at different time intervals. Data are shown as the mean ± SE of triplicate measurements from independent experiments. Statistical significance was assessed using a one-way ANOVA (analysis of variance, SPSS software v.18) test and the means were compared with Duncan's test at 0.05 level. [a-c] Accordingly, mean values with different small letters are considered statistically different (P≤0.05).

antimicrobial activity against indicator pathogen (20 ± 0.1 mm) as compared to the crude extract before acetone precipitation (15 ± 0.04 mm) (Figure 3).

The partially purified BLIS was subjected to FPLC-cation exchange chromatography on the SP-Sepharose column; the eluted fractions were lyophilized and tested for antimicrobial activity against pathogens to determine the active fractions (Figure 4). The fraction with antimicrobial activity was in the tail of the peak.

Molecular identification of the most potent isolate

The selected bacterial isolate (from fermented sausage) was similar to *L. plantarum,* with a similarity of 99.8%. The obtained sequence was submitted to GenBank as *L. plantarum* strain N1 under accession number OM019104.

Phylogenetic analysis with the alignment of the obtained gene sequences confirmed the belonging of the selected isolate to *Lactobacillus* genera, as shown in (Figure 5).

Development of biodegradable, antimicrobial packaging films using BLIS and determination of its activity

Different packaging films were made with different concentrations of cellulose acetate to determine the most suitable concentration for our study. The films that had sufficient flexibility suitable for food packaging were the films that had 3% and 6% cellulose acetate. The other concentrations were too rigid and difficult to handle, so they were unsuitable for food packaging. The thickness of the films was 300 µm. The 3% cellulose acetate films were coated with partially purified BLIS and tested for antimicrobial activity in triplicate using a disc diffusion assay. The coated films showed promising antimicrobial activity and inhibited the growth of the indicator pathogen *S. aureus* ATCC 29213 25 ± 0.2 mm. The control films (uncoated films) showed no inhibition zones against the indicator pathogen, as shown in (Figure 6).

DISCUSSION

Antimicrobial active packaging helps in the improvement of food safety by lowering the total count of microorganisms and the growth rate of microorganisms and/or extending the lag phase of microbial growth (Beigmohammadi *et al*., 2016; Fasihnia *et al*., 2018). Gutiérrez *et al*. (2011) evaluated the effect of antimicrobial active packaging on increasing the shelf-life of sliced bread and reported that the antimicrobial active packaging provided more microbiological inhibition and thus longer shelf-life than traditional packaging (not active). The usage of active packaging in the food industry may be more effective than the direct addition of bacteriocin as a preservative material in food and this is due to the controlled release of bacteriocin into the food matrix and reducing the probability of the inactivation of bacteriocin with food ingredients (Gumienna and Górna, 2021). Also, combining the protective function of the packaging and the antimicrobial activity of bacteriocins with other preservation methods, such as refrigeration

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

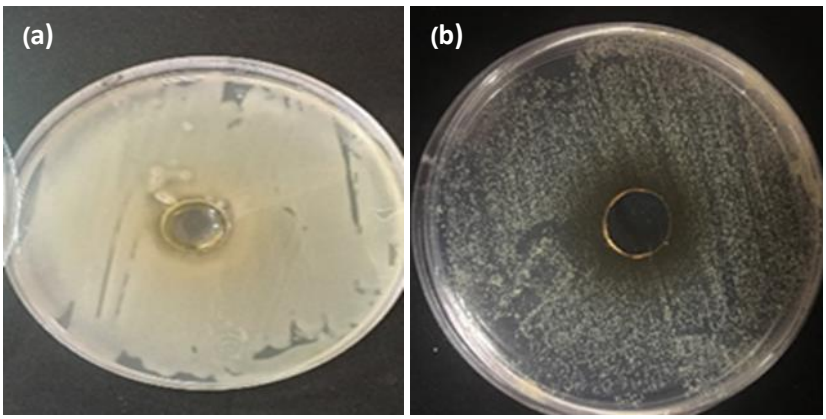

Figure 3: Antimicrobial activity of cell free supernatant produced from the most potent isolate (Strain N1) against *S. aureus* in triplicate. (a) Crude bacteriocin before acetone precipitation, (b) Partial purified bacteriocin after acetone precipitation.

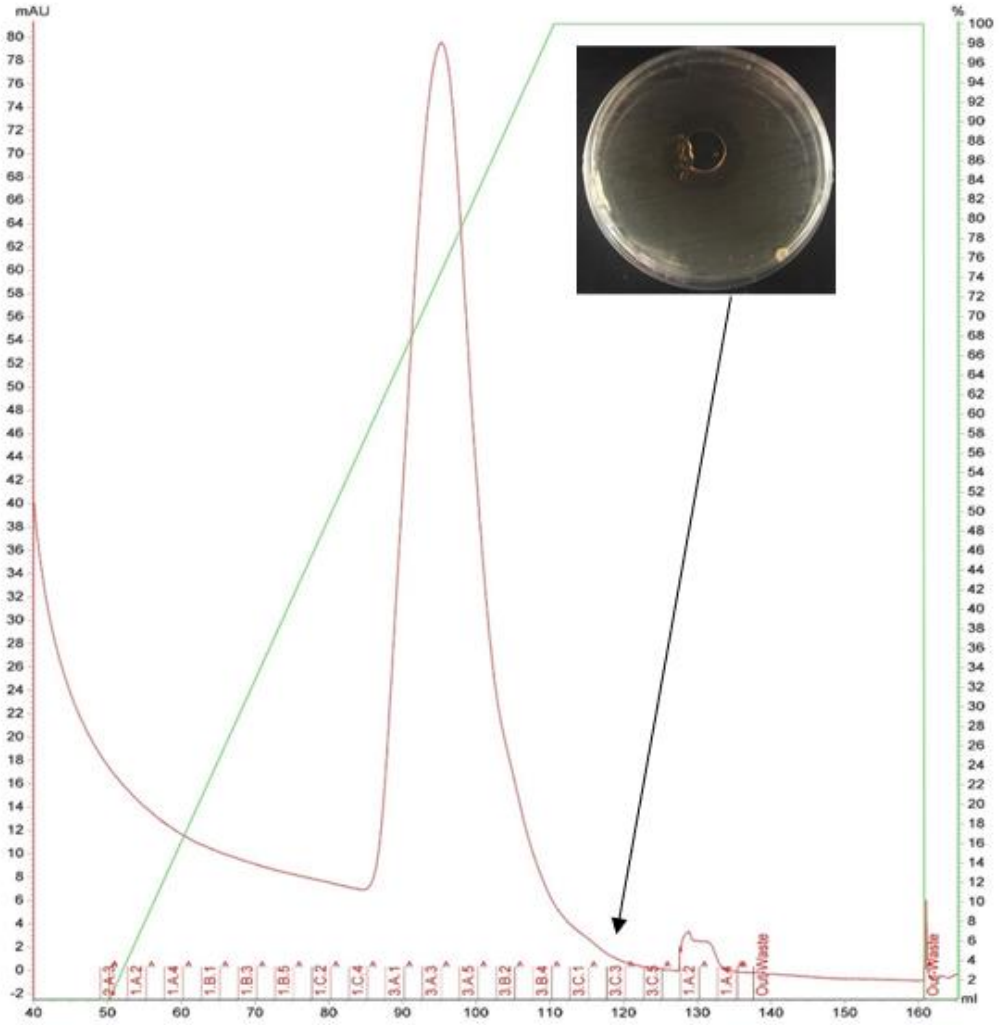

Figure 4: Purification of bacteriocin like inhibitory substance by using fast protein liquid chromatography-cation exchange chromatography and antimicrobial activity of active fraction (3C3).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 20(2) 2024, pp. 197-209
DOI: http://dx.doi.org/10.21161/mjm.230263

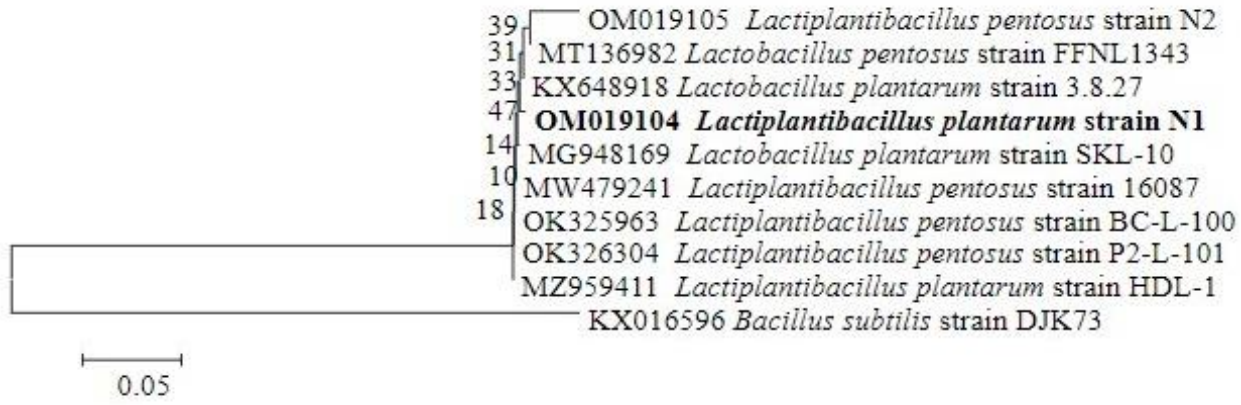

Figure 5: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of isolated strain and related reference taxa. The bar indicates 5% nucleotide substitution. Bold indicates the isolate identified in this study.

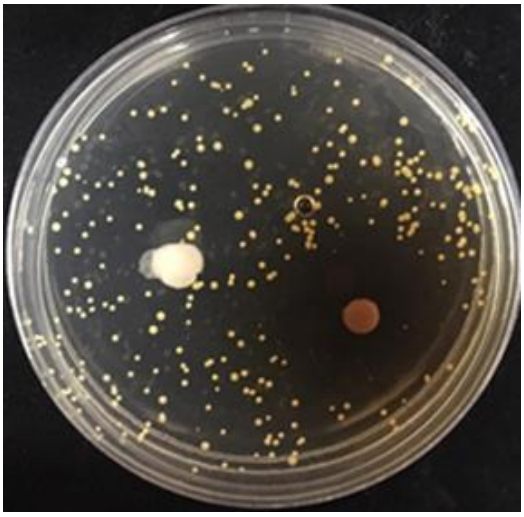

Figure 6: Antimicrobial activity of cellulose acetate film coated with bacteriocin-like inhibitory substance from *L. plantarum* strain N1 against *S. aureus* in triplicate. Control: Uncoated films without treatment.

and storage in a vacuum, would supply food products with better hygiene qualities (Iseppi *et al*., 2008).

In this study, a total of sixteen bacterial isolates were isolated from different local products from the Egyptian market. Several studies isolated LAB from the same sources in different regions (Rodríguez *et al*., 2000; Yang *et al*., 2012; Öz *et al*., 2017; Mıdık *et al*., 2020). According to the results of the biochemical tests, 10 out of 16 isolates were primarily identified as lactic acid bacteria. These ten isolates were Gram-positive, catalase-negative, and tolerated different levels of acidity and NaCl. This result came in accordance with Kumar *et al*. (2017), who characterized the lactic acid bacteria isolated from traditional pickles by using the same biochemical tests.

The result showed that the CFS of selected isolates showed antimicrobial activity against selected indicator pathogens with variable levels. The highest inhibition zone was detected against *S. aureus* ATCC 29213, which was selected for further studies. Several other researchers also reported the antimicrobial activity of bacteriocin and BLIS against *Staphylococcus* sp. (Hernández *et al.*, 2005; Zhu *et al*., 2014; Heredia-Castro *et al*., 2015; Islam *et al*., 2020), *P. aeruginosa (*Lv *et al*., 2018; Vataščinová *et al*., 2020), *S. mutans* ATCC 25175 (Wasfi *et al*., 2018) and *E. coli* (De Giani *et al*., 2019).

Based on the antimicrobial activity, one out of ten LAB isolates were selected as the most potent isolate (Strain N1). The CFS of this isolate was partially purified using the cold acetone precipitation method. Many researchers also used the cold acetone precipitation method to partially purify bacteriocin (Zendo *et al*., 2008; Yap *et al*., 2022). The antimicrobial activity of BLIS after acetone treatment (20 mm) was greater than the crude one (15 mm) and this is in agreement with Jamuna and Jeevaratnam (2004), who reported that bacteriocin from *Pediococcus* species showed higher activity after acetone precipitation.

The BLIS was purified using FPLC-cation exchange chromatography on an SP-Sephroase column. Chatterjee *et al*. (2021) also used FPLC-cation exchange chromatography to purify bacteriocin isolated from *Lactococcus lactis* JC10. The obtained fractions were tested for antimicrobial activity against the indicator pathogen (*S. aureus)*. The fraction that showed antimicrobial activity against the indicator pathogen was the 3C3 fraction, which was located in the tail of the peak; this might be due to very low concentrations of the protein. This is similar to the result reported by Zhang *et al*. (2018), who also used FPLC- cation exchange chromatography to purify a novel bacteriocin produced by *L. plantarum*. In Zhang's study, the active fraction was also revealed at the tail of the peak.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

There was a complete loss in the antimicrobial activity of the crude CFS after treatment with different proteolytic enzymes. At the same time, there was no loss in the antimicrobial activity after treatment with catalase enzyme, which suggests the proteinaceous nature of the inhibition substance responsible for CFS's antimicrobial activity and clearly excludes the involvement of H_2O_2 in the antimicrobial activity. Fernandes *et al*. (2017), Pei *et al*. (2020) and Mohapatra *et al.* (2021) also reported the proteinaceous nature of other bacteriocin extracted from different strains of *Lactobacillus plantarum.*

The extracted CFS was stable over a wide range of pH without a complete loss of antimicrobial activity at any tested pH value. This proved the pH stability of the obtained CFS. This is in agreement with Wang *et al*. (2018), who reported the pH stability of bacteriocin produced by *L. plantarum* isolated from fermented fish and also reported that bacteriocin maintained 90% of activity at pH 10, but in our study bacteriocin maintained 92.1% of activity at pH 10. And in agreement with another study by Lei *et al*. (2020) reported that the antimicrobial activity of bacteriocin obtained from *L. plantarum* zrx03 isolated from the infant's feces was stable in pH from 2 to 9.

The activity in case of heat treatment of CFS in this study is time-dependent; the activity significantly decreased when increasing the time of exposure at the same temperature. This is in agreement with Lu *et al*. (2020), who reported that the activity of novel bacteriocin produced by *Lactobacillus crustorum* MN047 decreased when increasing the time of exposure of each tested temperature (65 °C to 120 °C) for (5 min, 10 min and 20 min). BLIS maintained 95% of its activity even after exposure to 121 °C for 20 min (autoclaving); this is similar to Peng *et al*. (2021), who also reported novel bacteriocin produced by *L. plantarum* SHY 21-2 that maintained 95.8% of its activity even after treatment at 121 °C for 15 min. There are many studies that report the thermal stability of bacteriocin at different ranges of temperatures (Khalil *et al*., 2009; Zhang *et al*., 2018; Meng *et al*., 2021). This property would be useful in food industrial processing under pasteurization conditions. The thermal stability of bacteriocin and BLIS may be related to their molecular structure; they are usually composed of small peptides (Peng *et al*., 2021).

Over the past years, many bio-based polymers have been used in food packaging to avoid the environmental concerns caused by the excessive use of different synthetic polymers (El-Fawal, 2014). One of these bio-based polymers is cellulose acetate. Many studies used cellulose acetate to synthesize antimicrobial films to be used in food packaging (Gonçalves *et al*., 2020; Rajeswari *et al*., 2020).

In this study, cellulose acetate films were prepared by the casting method. Several concentrations of cellulose acetate were used (3% to 12%) to develop films. Only 3% and 6% concentrations were flexible and suitable for usage in food packaging; other concentrations produced rigid, difficult-to-handle films that were not suitable for food packaging. This is similar to Barbosa *et al*. (2013) who reported that 3% and 6% concentrations of cellulose acetate films were more flexible than other concentrations and more suitable to use as food packaging material.

Researchers reported different methods to develop active packaging. Some studies coated the films with bacteriocin (Kim *et al*., 2002; Bhattacharya *et al*., 2022), and others directly immobilized bacteriocin in the film matrix (Barbosa *et al*., 2013; Singh *et al.*, 2022; Zhang *et al*., 2023).

Samples from the treated films were tested for antimicrobial activity against indicator pathogens. The bacteriocin was diffused from the film to the agar and inhibited the growth around the film, and the films showed promising activity against the indicator pathogen *S. aureus* ATCC 29213 (25 ± 0.2 mm). Many other researchers reported the antimicrobial activity of films incorporated with bacteriocin as Scannell *et al*. (2000), who reported the antimicrobial activity of cellulose-based paper activated with Lacticin 3147 and Nisaplin. And La Storia *et al*. (2013) reported the antimicrobial activity of different commercial polyethylene films coated with nisin.

In this study, the developed cellulose acetate films have no antimicrobial activity, but the films acquired antimicrobial activity after coating with BLIS. This result is in agreement with El Fawal *et al*. (2019), who also evaluated the antimicrobial activity of cellulose acetate films incorporated with antimicrobial substances and reported that untreated cellulose films have no antimicrobial activity but acquired the activity after incorporated with rosemary and aloe vera essential oils as antimicrobial substances.

CONCLUSION

This study reported the production and characterization of BLIS extracted from *L. plantarum* strain N1. The BLIS was purified using a two-step protocol: acetone precipitation and SP-Sephroase cation exchange chromatography. The produced BLIS was stable over a wide range of temperatures and pH. This study succeeded in developing active antimicrobial packaging by incorporating cellulose acetate films with partially purified BLIS. These films showed promising antimicrobial activity against *S. aureus* ATCC 29213.

AUTHOR CONTRIBUTIONS

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Noha A. Ali and Ali M. Saeed. The first draft of the manuscript was written by Noha A. Ali and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

FUNDING

This study was funded by the Academy of Scientific Research and Technology (ASRT) in Egypt as a part of

the scholarship Scientists of Next Generation (SNG) cycle 6.

ACKNOWLEDGEMENTS

This study is funded by the Academy of Scientific Research and Technology (ASRT) in Egypt as a part of the scholarship Scientists of Next-Generation (SNG) cycle 6. The authors are greatly thankful to ASRT, the Department of Microbiology, Faculty of Science, Ain Shams University, Egypt and Food Technology Research Institute, Agriculture Research Center.

CONFLICTS OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

REFERENCES

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. *et al.* (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research* **25(17), 3389-3402.**

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D. and Kuipers, O. P. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Applied Microbiology and Biotechnology* **100, 2939-2951.**

Amin, U., Khan, M. K. I., Maan, A. A., Nazir, A., Riaz, S., Khan, M. U. *et al.* (2022). Biodegradable active, intelligent, and smart packaging materials for food applications. *Food Packaging and Shelf Life* **33, 100903.**

Amit, S. K., Uddin, M. M., Rahman, R., Islam, S. M. R. and Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. *Agriculture and Food Security* **6, 51.**

Assis, R. Q., Pagno, C. H., Stoll, L., Rios, P. D., de Oliveira Rios, A. and Olivera, F. C. (2021). Active food packaging of cellulose acetate: Storage stability, protective effect on oxidation of riboflavin and release in food simulants. *Food Chemistry* **349, 129140.**

Barbosa, A. A. T., de Araújo, H. G. S., Matos, P. N., Carnelossi, M. A. G. and de Castro, A. A. (2013). Effects of nisin-incorporated films on the microbiological and physicochemical quality of minimally processed mangoes. *International Journal of Food Microbiology* **164(2-3), 135-140.**

Beigmohammadi, F., Peighambardoust, S. H., Hesari, J., Azadmard-Damirchi, S., Peighambardoust, S. J. and Khosrowshahi, N. K. (2016). Antibacterial properties of LDPE nanocomposite films in packaging of UF cheese. *LWT - Food Science and Technology* **65, 106-111.**

Bhattacharya, D., Nanda, P. K., Pateiro, M., Lorenzo, J. M., Dhar, P. and Das, A. K. (2022). Lactic acid bacteria and bacteriocins: Novel biotechnological approach for biopreservation of meat and meat products. *Microorganisms* **10(10), 2058.**

Bostan, K., Alcay, A. U., Yalçin, S., Vapur, U. E. and Nizamlioglu, M. (2017). Identification and characterization of lactic acid bacteria isolated from traditional cone yoghurt. *Food Science and Biotechnology* **26(6), 1625-1632.**

Chatterjee, M., Jana, S. C. and Raychaudhuri, U. (2021). Isolation, purification and characterization of a bacteriocin with broad spectrum activity from *Lactococcus lactis* JC10 from perishable papaya fruit. *Journal of Microbiology, Biotechnology and Food Sciences* **6(1), 655-660.**

Contessa, C. R., da Rosa, G. S. and Moraes, C. C. (2021). New active packaging based on biopolymeric mixture added with bacteriocin as active compound. *International Journal of Molecular Sciences* **22(19), 10628.**

Contessa, C. R., de Souza, N. B., Gonçalo, G. B., de Moura, C. M., da Rosa, G. S. and Moraes, C. C. (2021). Development of active packaging based on agar-agar incorporated with bacteriocin of *Lactobacillus sakei*. *Biomolecules* **11(12), 1869.**

De Giani, A., Bovio, F., Forcella, M., Fusi, P., Sello, G. and Di Gennaro, P. (2019). Identification of a bacteriocin-like compound from *Lactobacillus plantarum* with antimicrobial activity and effects on normal and cancerogenic human intestinal cells. *AMB Express* **9, 88.**

El-Fawal, G. (2014). Preparation, characterization and antibacterial activity of biodegradable films prepared from carrageenan. *Journal of Food Science and Technology* **51, 2234-2239.**

El Fawal, G. F., Omer, A. M. and Tamer, T. M. (2019). Evaluation of antimicrobial and antioxidant activities for cellulose acetate films incorporated with rosemary and aloe vera essential oils. *Journal of Food Science and Technology* **56, 1510-1518.**

Fasihnia, S. H., Peighambardoust, S. H. and Peighambardoust, S. J. (2018). Nanocomposite films containing organoclay nanoparticles as an antimicrobial (active) packaging for potential food application. *Journal of Food Processing and Preservation* **42(2), e13488.**

Fernandes, A. and Jobby, R. (2022). Bacteriocins from lactic acid bacteria and their potential clinical applications. *Applied Biochemistry and Biotechnology* **194(10), 4377-4399.**

Fernandes, P., Loureiro, D., Monteiro, V., Ramos, C., Nero, L. A., Todorov, S. D. *et al.* (2017). *Lactobacillus plantarum* isolated from cheese: Production and partial characterization of bacteriocin B391. *Annals of Microbiology* **67(6), 433-442.**

Giello, M., Volpe, S., Sequino, G., De Filippis, F., Villani, F. and Torrieri, E. (2023). Design of bioactive biopolymer coating based on *Latilactobacillus curvatus* 54M16 producer of bacteriocins to preserve the safety of minimally processed fennel. *Food Packaging and Shelf Life* **38, 101111.**

Gonçalves, S. M., Motta, J. F. G., Ribeiro-Santos, R., Chávez, D. W. H. and de Melo, N. R. (2020). Functional and antimicrobial properties of cellulose

acetate films incorporated with sweet fennel essential oil and plasticizers. *Current Research in Food Science* **3, 1-8.**

Gouvêa, D. M., Mendonça, R. C. S., Soto, M. L. and Cruz, R. S. (2015). Acetate cellulose film with bacteriophages for potential antimicrobial use in food packaging. *LWT - Food Science and Technology* **63(1), 85-91.**

Gumienna, M. and Górna, B. (2021). Antimicrobial food packaging with biodegradable polymers and bacteriocins. *Molecules* **26(12), 3735.**

Gutiérrez, L., Batlle, R., Andújar, S., Sánchez, C. and Nerín, C. (2011). Evaluation of antimicrobial active packaging to increase shelf life of gluten-free sliced bread. *Packaging Technology and Science* **24(8), 485-494.**

Heredia-Castro, P. Y., Méndez-Romero, J. I., Hernández-Mendoza, A., Acedo-Félix, E., González-Córdova, A. F. and Vallejo-Cordoba, B. (2015). Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances produced by *Lactobacillus* spp. isolated from artisanal Mexican cheese. *Journal of Dairy Science* **98(12), 8285-8293.**

Hernández, D., Cardell, E. and Zárate, V. (2005). Antimicrobial activity of lactic acid bacteria isolated from Tenerife cheese: Initial characterization of plantaricin TF711, a bacteriocin-like substance produced by *Lactobacillus plantarum* TF711. *Journal of Applied Microbiology* **99(1), 77-84.**

Huang, T., Qian, Y., Wei, J. and Zhou, C. (2019). Polymeric antimicrobial food packaging and its applications. *Polymers* **11(3), 560.**

Imran, M., El-Fahmy, S., Revol-Junelles, A. and Desobry, S. (2010). Cellulose derivative based active coatings: Effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films. *Carbohydrate Polymers* **81(2), 219-225.**

Iseppi, R., Pilati, F., Marini, M., Toselli, M., de Niederhäusern, S., Guerrieri, E. *et al.* (2008). Anti-listerial activity of a polymeric film coated with hybrid coatings doped with Enterocin 416K1 for use as bioactive food packaging. *International Journal of Food Microbiology* **123(3), 281-287.**

Islam, M. Z., Uddin, M. E., Rahman, M. T., Islam, M. A. and Harun-ur-Rashid, M. (2021). Isolation and characterization of dominant lactic acid bacteria from raw goat milk: Assessment of probiotic potential and technological properties. *Small Ruminant Research* **205, 106532.**

Islam, R., Hossain, M. N., Alam, M. K., Uddin, M. E., Rony, M. H., Imran, M. A. S. *et al.* (2020). Antibacterial activity of lactic acid bacteria and extraction of bacteriocin protein. *Advances in Bioscience and Biotechnology* **11(2), 49-59.**

Jamuna, M. and Jeevaratnam, K. (2004). Isolation and partial characterization of bacteriocins from *Pediococcus* species. *Applied Microbiology and Biotechnology* **65(4), 433-439.**

Khalil, R., Elbahloul, Y., Djadouni, F. and Omar, S. (2009). Isolation and partial characterization of a bacteriocin produced by a newly isolated *Bacillus megaterium* 19 strain. *Pakistan Journal of Nutrition* **8(3), 242-250.**

Kim, Y. M., Paik, H. D. and Lee, D. S. (2002). Shelf-life characteristics of fresh oysters and ground beef as affected by bacteriocin-coated plastic packaging film. *Journal of the Science of Food and Agriculture* **82(9), 998-1002.**

Kumar, V., Kumari, A., Angmo, K. and Bhalla, T. C. (2017). Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India. *Journal of Food Science and Technology* **54, 1945-1952.**

La Storia, A., Ercolini, D., Marinello, F. and Mauriello, G. (2008). Characterization of bacteriocin-coated antimicrobial polyethylene films by atomic force microscopy. *Journal of Food Science* **73(4), T48-T54.**

La Storia, A., Mauriello, G., Villani, F. and Ercolini, D. (2013). Coating-activation and antimicrobial efficacy of different polyethylene films with a nisin-based solution. *Food and Bioprocess Technology* **6, 2770-2779.**

Lei, S., Zhao, R., Sun, J., Ran, J., Ruan, X. and Zhu, Y. (2020). Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. *Food Science and Nutrition* **8(5), 2214-2222.**

Lu, Y., Aizhan, R., Yan, H., Li, X., Wang, X., Yi, Y. *et al.* (2020). Characterization, modes of action, and application of a novel broad-spectrum bacteriocin BM1300 produced by *Lactobacillus crustorum* MN047. *Brazilian Journal of Microbiology* **51, 2033-2048.**

Lv, X., Lin, Y., Jie, Y., Sun, M., Zhang, B., Bai, F. *et al.* (2018). Purification, characterization, and action mechanism of plantaricin DL3, a novel bacteriocin against *P. aeruginosa* produced by *Lactobacillus plantarum* DL3 from Chinese Suan-Tsai. *European Food Research and Technology* **244(2), 323-331.**

Manzoor, A., Yousuf, B., Pandith, J. A. and Ahmad, S. (2023). Plant-derived active substances incorporated as antioxidant, antibacterial or antifungal components in coatings/films for food packaging applications. *Food Bioscience* **53, 102717.**

Marrez, D. A., Abdelhamid, A. E. and Darwesh, O. M. (2019). Eco-friendly cellulose acetate green synthesized silver nano-composite as antibacterial packaging system for food safety. *Food Packaging and Shelf Life* **20, 100302.**

Marvdashti, L. M., Koocheki, A. and Yavarmanesh, M. (2019). Characterization, release profile and antimicrobial properties of bioactive polyvinyl alcohol-*Alyssum homolocarpum* seed gum-nisin composite film. *Food Biophysics* **14, 120-131.**

Meng, F., Zhu, X., Zhao, H., Nie, T., Lu, F., Lu, Z. *et al.* (2021). A class III bacteriocin with broad-spectrum antibacterial activity from *Lactobacillus acidophilus* NX2-6 and its preservation in milk and cheese. *Food Control* **121, 107597.**

Mıdık, F., Tokatlı, M., Elmacı, S. B. and Özçelik, F. (2020). Influence of different culture conditions on exopolysaccharide production by indigenous lactic acid bacteria isolated from pickles. *Archives of Microbiology* **202, 875-885.**

Mohapatra, A. R., Lakshmanan, D., Mahesh, R., Suchiang, K. and Jeevaratnam, K. (2021). Characterization and cytotoxic evaluation of bacteriocins possessing antibiofilm activity produced by *Lactobacillus plantarum* SJ33. *International Journal of Peptide Research and Therapeutics* **27, 1783-1797.**

O'Connor, P. M., Kuniyoshi, T. M., Oliveira, R. P., Hill, C., Ross, R. P. and Cotter, P. D. (2020). Antimicrobials for food and feed; A bacteriocin perspective. *Current Opinion in Biotechnology* **61, 160-167.**

Öz, E., Kaban, G., Barış, Ö. and Kaya, M. (2017). Isolation and identification of lactic acid bacteria from pastırma. *Food Control* **77, 158-162.**

Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E. and Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science* **65, 859-867.**

Pei, J., Jin, W., Abd El-Aty, A. M., Baranenko, D. A., Gou, X., Zhang, H. *et al.* (2020). Isolation, purification, and structural identification of a new bacteriocin made by *Lactobacillus plantarum* found in conventional kombucha. *Food Control* **110, 106923.**

Peng, S., Song, J., Zeng, W., Wang, H., Zhang, Y., Xin, J. *et al.* (2021). A broad-spectrum novel bacteriocin produced by *Lactobacillus plantarum* SHY 21-2 from yak yogurt: Purification, antimicrobial characteristics and antibacterial mechanism. *LWT* **142, 110955.**

Rajeswari, A., Christy, E. J. S., Swathi, E. and Pius, A. (2020). Fabrication of improved cellulose acetate-based biodegradable films for food packaging applications. *Environmental Chemistry and Ecotoxicology* **2, 107-114.**

Rea, M. C., Ross, R. P., Cotter, P. D. and Hill, C. (2011). Classification of bacteriocins from Gram-positive bacteria. *In*: Prokaryotic Antimicrobial Peptides: From Genes to Applications. Drider, D. and Rebuffat, S. (eds.). Springer, New York. **pp. 29-53.**

Rodríguez, E., González, B., Gaya, P., Nuñez, M. and Medina, M. (2000). Diversity of bacteriocins produced by lactic acid bacteria isolated from raw milk. *International Dairy Journal* **10(1-2), 7-15.**

Rossi, E., Ali, A., Efendi, R., Restuhadi, F., Zalfiatri, Y., Sofyan, Y. *et al.* (2021). Characterization of bacteriocin produced by lactic acid bacteria isolated from solid waste of soymilk production. *IOP Conference Series: Earth and Environmental Science* **709, 012020.**

Saavedra, L. and Sesma, F. (2011). Purification techniques of bacteriocins from lactic acid bacteria and other Gram-positive bacteria. *In*: Prokaryotic Antimicrobial Peptides: From Genes to Applications. Drider, D. and Rebuffat, S. (eds.). Springer, New York. **pp. 99.**

Sadeghi, A., Katouzian, I., Ebrahimi, M., Assadpour, E., Tan, C. and Jafari, S. M. (2023). Bacteriocin-like inhibitory substances as green bio-preservatives; nanoliposomal encapsulation and evaluation of their *in vitro*/*in situ* anti-Listerial activity. *Food Control* **150, 109725.**

Salvucci, E., Rossi, M., Colombo, A., Pérez, G., Borneo, R. and Aguirre, A. (2019). Triticale flour films added with bacteriocin-like substance (BLIS) for active food packaging applications. *Food Packaging and Shelf Life* **19, 193-199.**

Santiago-Silva, P., Soares, N. F., Nóbrega, J. E., Júnior, M. A., Barbosa, K. B., Volp, A. C. P. *et al.* (2009). Antimicrobial efficiency of film incorporated with pediocin (ALTA® 2351) on preservation of sliced ham. *Food Control* **20(1), 85-89.**

Scannell, A. G., Hill, C., Ross, R. P., Marx, S., Hartmeier, W. and Arendt, E. K. (2000). Development of bioactive food packaging materials using immobilised bacteriocins Lacticin 3147 and Nisaplin®. *International Journal of Food Microbiology* **60(2-3), 241-249.**

Sidhu, P. K. and Nehra, K. (2021). Purification and characterization of bacteriocin Bac23 extracted from *Lactobacillus plantarum* PKLP5 and its interaction with silver nanoparticles for enhanced antimicrobial spectrum against food-borne pathogens. *LWT* **139, 110546.**

Singh, G. P., Bangar, S. P., Yang, T., Trif, M., Kumar, V. and Kumar, D. (2022). Effect on the properties of edible starch-based films by the incorporation of additives: A review. *Polymers* **14(10), 1987.**

Sultan, K. A. Y. A. and Hatice, K. A. T. I. (2023). Biochemical characterization and partial purification of a bacteriocin like-inhibitory substance produced from *Bacillus* sp. T68 Strain. *Eskişehir Technical University Journal of Science and Technology A - Applied Sciences and Engineering* **24(1), 1-13.**

Sun, H., Shao, X., Zhang, M., Wang, Z., Dong, J. and Yu, D. (2019). Mechanical, barrier and antimicrobial properties of corn distarch phosphate/nanocrystalline cellulose films incorporated with Nisin and ε-polylysine. *International Journal of Biological Macromolecules* **136, 839-846.**

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30(12), 2725-2729.**

Tumbarski, Y., Deseva, I., Mihaylova, D., Stoyanova, M., Krastev, L., Nikolova, R. *et al.* (2018). Isolation, characterization and amino acid composition of a bacteriocin produced by *Bacillus methylotrophicus* strain BM47. *Food Technology and Biotechnology* ***56*(4), 546-552.**

Vataščinová, T., Pipová, M., Fraqueza, M. J. R., Maľa, P., Dudriková, E., Drážovská, M. *et al.* (2020). Antimicrobial potential of *Lactobacillus plantarum* strains isolated from Slovak raw sheep milk cheeses. *Journal of Dairy Science* **103(8), 6900-6903.**

Verma, D. K., Thakur, M., Singh, S., Tripathy, S., Gupta, A. K., Baranwal, D. *et al.* (2022). Bacteriocins as antimicrobial and preservative agents in food: Biosynthesis, separation and application. *Food Bioscience* **46, 101594.**

Villalobos-Delgado, L. H., Nevárez-Moorillon, G. V., Caro, I., Quinto, E. J. and Mateo, J. (2019). Natural antimicrobial agents to improve foods shelf life. *In*: Food Quality and Shelf Life. Galanakis, C. M. (ed.). Academic Press, United Kingdom. **pp. 125-157.**

Wang, Y., Qin, Y., Xie, Q., Zhang, Y., Hu, J. and Li, P. (2018). Purification and characterization of plantaricin LPL-1, a novel class IIa bacteriocin produced by *Lactobacillus plantarum* LPL-1 isolated from fermented fish. *Frontiers in Microbiology* **9, 2276.**

Wasfi, R., Abd El-Rahman, O. A., Zafer, M. M. and Ashour, H. M. (2018). Probiotic *Lactobacillus* sp. inhibit growth, biofilm formation and gene expression of caries-inducing *Streptococcus mutans*. *Journal of Cellular and Molecular Medicine* **22(3), 1972-1983.**

Woraprayote, W., Pumpuang, L., Tosukhowong, A., Zendo, T., Sonomoto, K., Benjakul, S. *et al.* (2018). Antimicrobial biodegradable food packaging impregnated with Bacteriocin 7293 for control of pathogenic bacteria in pangasius fish fillets. *LWT* ***89*****, 427-433.**

Yang, E., Fan, L., Jiang, Y., Doucette, C. and Fillmore, S. (2012). Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express* **2(1), 48.**

Yang, Y., Liu, H., Wu, M., Ma, J. and Lu, P. (2020). Bio-based antimicrobial packaging from sugarcane bagasse nanocellulose/nisin hybrid films. *International Journal of Biological Macromolecules* **161, 627-635.**

Yap, P. G., Lai, Z. W. and Tan, J. S. (2022). Bacteriocins from lactic acid bacteria: Purification strategies and applications in food and medical industries: A review. *Beni-Suef University Journal of Basic and Applied Sciences* **11, 51.**

Zendo, T., Nakayama, J., Fujita, K. and Sonomoto, K. (2008). Bacteriocin detection by liquid chromatography/mass spectrometry for rapid identification. *Journal of Applied Microbiology* **104(2), 499-507.**

Zhang, A., Han, Y. and Zhou, Z. (2023). Characterization of citric acid crosslinked chitosan/gelatin composite film with enterocin CHQS and red cabbage pigment. *Food Hydrocolloids* **135, 108144.**

Zhang, J., Yang, Y., Yang, H., Bu, Y., Yi, H., Zhang, L. *et al.* (2018). Purification and partial characterization of bacteriocin Lac-B23, a novel bacteriocin production by *Lactobacillus plantarum* J23, isolated from Chinese traditional fermented milk. *Frontiers in Microbiology* **9, 2165.**

Zhang, M., Gao, X., Zhang, H., Liu, H., Jin, J., Yang, W. *et al.* (2017). Development and antilisterial activity of PE-based biological preservative films incorporating plantaricin BM-1. *FEMS Microbiology Letters* **364(7), fnw283.**

Zhang, Y., Tang, Q., Huang, K., Xu, Z., Feng, S., Li, H. *et al.* (2022). Developing strong and tough cellulose acetate/ZIF67 intelligent active films for shrimp freshness monitoring. *Carbohydrate Polymers* **302, 120375.**

Zhu, X., Zhao, Y., Sun, Y. and Gu, Q. (2014). Purification and characterisation of plantaricin ZJ008, a novel bacteriocin against *Staphylococcus* spp. from *Lactobacillus plantarum* ZJ008. *Food Chemistry* **165, 216-223.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538