International Journal of Endorsing
HEALTH SCIENCE RESEARCH

IJEHSR

Original Article

Isolation and characterization of bacteriocin-like inhibitory substances producing lactic acid bacteria from indigenous food samples.

Nayyar Mehmood, Abdul Wahab, Urooba Jamal, Waseem Ahmed Mangi, Syed Abdus Subhan & Tanveer Abbas
Department of Microbiology, University of Karachi, Karachi-Pakistan.

Doi: 10.29052/IJEHSR.v10.i3.2022.328-336
Corresponding Author Email: taabbas@uok.edu.pk
Received 02/02/2022
Accepted 30/05/2022
First Published 22/06/2022

OPEN ACCESS

Abstract

Background: Lactic acid bacteria (LAB) are generally found in nutrient-rich environments such as milk, dairy products, meat, vegetables, and fruits. The functions of LAB depend upon the sufficient number of bacteria being available in the intestines, and the actions of these bacteria are generally species and strain-specific. The challenges encountered in identifying and classifying lactic acid bacteria have complicated the research process. Despite encountered challenges, various benefits of lactic acid bacteria have been identified. The current study aimed to isolate and characterize indigenous LAB bacteriocins producers from local food sources using morphological, biochemical, and molecular methods.

Methodology: One thousand indigenous LAB were isolated and screened from local fresh and fermented foods in Karachi's retail markets for three years. 46 LAB isolates exhibited inhibitory activity against other LAB and non-LAB gram-positive bacteria in broth medium. Three of forty-six isolates were selected for further study of the nature and production of the inhibitory substance based on production in broth, bacteriocin nature of the substance, and relatively wide antibacterial spectrum.

Results: The three indigenous LAB producers were identified as Lactococci based on microscopy, catalase, biochemical and molecular characterization. Bacteriocin of the isolate C130 was heat tolerant at 100°C and produced during the late logarithmic phase of growth. There is still a need for investigation to identify the role of LAB, which might help prevent certain chronic illnesses and infections. Most beneficial impacts are known in constipation, hypocholesterolemic effects, urogenital infections, and colon cancer.

Conclusion: L. lactis C130 was found to be a thermostable bacteriocin producer with a fair antibacterial spectrum against various food spoilage.

Keywords

Lactic Acid Bacteria, Bacteriocin-Like Inhibitory Substances, Indigenous Food Samples.

Check for updates

Introduction

Lactic acid bacteria (LAB) is a group of Gram-positive cocci or bacilli, catalase-negative, non-sporing, and produced lactic acid by fermentation of carbohydrate[1]. This LAB group consists of several genera, including Lactococcus, Lactobacillus, Leuconostoc, Enterococcus, Carnobacterium, Aerococcus, Pediococcus, Oenococcus, Streptococcus, Tetragonococcus, Vagococcus, and Weisellacoccus[2]. Apart from carbohydrate fermentation, LAB can break down proteins and fats present in food and helps in good absorption of food that's LAB also behave like probiotics[3]. LAB, in fermented foods, are generally regarded as safe (GRAS) bacteria[4]. Their metabolic end products are responsible for flavor, aroma, and texture and also improve the shelf life of these products as humans have consumed these metabolites for many centuries without any adverse effects. These products in the fermentation industry include lactic acid, acetic acid, diacetyl, hydrogen peroxide, and many other volatile compounds[5]. These products are responsible for the active inhibition of spoilage and pathogenic bacteria in traditional fermented foods worldwide[6,7]. Another active inhibitory compound of LAB is bacteriocin, identified as ribosomally synthesized proteins/peptide substances produced by bacteria that kill or inhibit the growth of other closely related bacteria[8].

It is estimated that most bacteria produce some kind of BLIS[9]. BLIS differs from antibiotics by having spectra of antimicrobial activity against various microorganisms[10]. Bacteriocins of LAB are classified into four classes based on their structure and genetic characteristics. Class, I bacteriocins are also called lantibiotics; they are small, heat-stable peptides. Class II bacteriocins are non-lanthionine, heat stable, small peptides. Class III bacteriocins are large heat-labile proteins, and class IV bacteriocins are complex heat-labile protein conjugates[11,12]. Bacteriocins of LAB are important in the food fermentation industry because LAB starters can produce these bacteriocins in situ. Moreover, their purified or semi-purified bacteriocins are added to food as preservatives[13].

Nysain A/Z and pediocin 1 are commercial bacteriocins approved as food additives and are used in most foods[14]. LAB bacteriocins, being protein in nature, can be degraded by proteases of the gastrointestinal tract and are therefore believed to be safe for human consumption[15]. Bacteriocin production in LAB is influenced by many environmental factors such as temperature, pH, and nutrients[16]. Bacteriocin production in culture shows mixed metabolic kinetics, and it is reported to be produced in exponential and stationary phases of growth[17]. They are also important metabolites that are used in food preservation as well as in healthcare[18,19].

This study aims to isolate and characterize indigenous LAB bacteriocins producers from local food sources using morphological, biochemical, and molecular methods. After these characterizations, these LAB isolates become a potential culture for lactic acid starter culture in food and dairy.

Methodology

Different food samples, including vegetables, raw milk, yogurt, and cheese, were purchased from local retail markets in Karachi. These food samples were screened over three years to isolate indigenous lactic acid bacteria.

Determination of CFU/g or CFU/ml of food sample

Indigenous LAB was isolated from each sample, as shown in Table 1. The isolate was purified by MRS[20] agar plate streaking, inoculation in MRS broth, microscopy, Gram reaction, and catalase production.

Table 1 (a): Overall production of Bacteriocin Inhibitory substances from indigenous LAB isolates.

Sample	Number of BLIS producing LAB isolates produced Z.O.I	Production of BLIS in broth by Z.O.I producers LAB isolates	Bacteriocin producers / BLIS producers
Vegetable	60	15	02/15
Raw Milk	40	9	02/09
Yogurt	80	7	02/07
Cheese	100	15	05/15

*All LAB Isolates Were Gram's Reaction Positive and Catalase Negative; C.F.S. = Cell-Free Supernatant, Protease-Sensitive, Acid Neutralized; Z.O.I. = Zone Of Inhibition

Table 1(b): Indigenous Lactic Acid Bacteria Bacteriocin producers from food/dairy sources with their spectrum of activity.

Bacteriocin Producers from food/dairy sources	LAB Genus	Activity Spectrum	Percentage Bacteriocin producers (bacteriocin producers/BLIS producers)
V63	Lactobacilli	Narrow	13.33(02/15)
V79	Lactobacilli	Narrow	13.33(02/15)
M132	Lactococci	Narrow	22.22(02/09)
M158	Lactococci	Narrow	22.22(02/09)
Y54	Lactobacilli	Narrow	28.57(02/07)
Y71	Lactococci	Narrow	28.57(02/07)
C27	Lactococci	Wide	33.33(05/15)
C100	Lactococci	Narrow	33.33(05/15)
C130	Lactococci	Wide	33.33(05/15)
C236	Lactococci	Wide	33.33(05/15)
C240	Lactobacilli	Narrow	33.33(05/15)

Bacteriocin Producers: V= vegetable isolate, M= Milk isolate, Y= Yogurt isolate, C= Cheese isolate

Isolation of inhibitor-producing LAB

To determine bacteriocin activity of indigenous LAB[21] antagonistic assays were performed using Lactococcus lactis and Lactobacillus casei as indicators. A total of one thousand Indigenous LAB isolates from all of the four samples (250 from each sample, i.e., vegetable, milk, yogurt, and cheese) were randomly picked and transferred to freshly prepared MRS agar plates and incubated overnight at 30°C. 6 ml of MRS agar containing a fresh culture of the indicator was poured over the agar plate. The plate was further incubated overnight at 30°C and observed for a zone of inhibition.

Production of Inhibitory Activity in broth

Purified inhibitor-producing isolates from the solid plate were inoculated in 100 ml MRS broth for 24 h at 30°C to produce the inhibitory substance.

Preparation of cell-free supernate (CFS)

24 h broth culture of inhibitor producer were centrifuged at 10,000 rpm for 10 minutes; the supernate was filter-sterilized using a 0.22 µm filter. It was adjusted to pH 6.5 using 1M NaOH.

Spot–on–lawn technique

This technique was used to check the inhibitory Activity of CFS[22]. CFS was spotted on the agar surface containing fresh indicator lawn and incubated at 30°C overnight for inhibition zone production.

Sensitivity of inhibitory activity to protease

1 ml mixture of CFS with proteinase K (Sigma) 1 mg/ml was incubated at 37°C for 3 hrs. Then CFS was checked for arbitrary units AU/ml, compared to control CFS with no protease treatment.

Bacteriocin Bioassay

Critical dilution method[23] was used to measure AU/ml in CFS. Two-fold serial dilution of CFS was checked for inhibition zones as described above. Bacteriocin activity was calculated based on the formula; AU ml^{-1}= 2^n × 1000/20 (where n is the number of times dilution is made).

Temperature stability tests of bacteriocin preparations

3 ml CFS, at pH 6.5 of bacteriocin producers, were treated at 50°C, 60°C, 70°C, 80°C, and 100°C (water bath) for different time intervals. After cooling, AU ml^{-1} of bacteriocin was determined as compared to control CFS (no heat treatment) given in Table 2.

Table 2: Effect of Protease, heat treatment, and pH on the activity of bacteriocins of selected LAB producers.

Variables		Residual Activity in Arbitrary Units/ml		
		C-27	C-130	C-236
Protease		0	0	0
Heat for 30 min	50°C	800	1600	800
	60°C	800	1600	0
	80°C	0	800	0
	100°C	0	400	0
pH for 8 h	2	1600	1600	1600
	4	1600	1600	1600
	5	1600	1600	1600
	6	1600	1600	1600
	7	1600	1600	1600
	8	0	400	0
	9	0	0	0
Control (No treatment)		1600	1600	1600

Table 3: Inhibitory spectrum of bacteriocin producers.

Indicators	Bacteriocin Producers		
	C-27	C-130	C-236
Lactococcus lactis	++	++	++
Lactobacillus casei	-	-	-
Bacillus cereus	-	++	-
Corynebacterium hofmanii	++	++	+
Staphylococcus aureus	+	++	+
Streptococcus faecalis	+	+	-
Micrococcus flavus	++	++	++
Escherichia coli	-	-	-
Pseudomonas aeruginosa	-	-	-
Salmonella typhi	-	-	-

(+) Less activity, (++) More activity, (-) No activity

pH stability

the pH of CFS of bacteriocin producers was set in the range of 2 to 9 by 2M HCl or NaOH solution. After 24 h at room temperature, pH was changed to 6.5, and AU/ml was determined compared to control CFS (no pH treatment).

Determination of bacteriocin inhibitory spectrum

Different food spoilage bacteria and foodborne pathogens (Table 3) were used to check the spectrum of bacteriocin inhibition present in CFS. Tryptone soy agar (Oxoid) was used to grow non-LAB bacteria.

Physiological & biochemical characterization of bacteriocin-producing strain

Carbohydrate fermentation profiles and biochemical characteristics of bacteriocin producers LAB were determined using API 20 Strep for lactococci, streptococci, and enterococci (Biomerieux, France), according to the manufacturer's protocol.

Genetic identification of bacteriocin-producing LAB strains

Molecular identification of LAB strains was carried out by PCR with species-specific primers, listed below, using Lactococcus lactis as a reference organism with gene bank accession number 'FJ 795655'. Three sets of primers used in this study are:

F1:AATTTGAAGAGCAGCGAACGGGTG;
F2:ACGTTGGTGAGAGTGGAAAGCTCA;
F3:AGCGTTGTCCGGATTTATTG;
R1:ATGTATCATCGCCTTGGTGAGCCT;
R2:AACGTTCTTCTCACCAAC;
R3:TGCCGTTAGCTGCGATACAGAGAA;

with denaturation temperature 94°C for 4 mins for 1st cycle than for 1 min for 35 cycles, annealing temperature 56°C for 1 min, extension temperature 72°C for 1 min, last extension for 5 mins and then hold at 4°C. 25 µl PCR reactions were prepared using a Green GoTaq master mix kit (Promega) according to manufacture protocols. 5 µl PCR products were then analyzed by 1% agarose gel electrophoresis.

Bacteriocin production during growth

One liter of MRS broth was inoculated with a 2% v/v overnight culture of C130 and incubated at 30°C. The growth was measured turbidometrically at 30°C. Aliquots (3ml) were collected from cultured broth at several time points for growth (OD 600 nm) and bacteriocin (in CFS).

Results

Lactic acid bacterial flora of the food samples

All of these LAB were gram-positive, catalase-negative, non-sporing lactococci, and bacilli (Table 1a and 1b) present in all four food sample groups. 250 LAB isolates from each sample were randomly picked to give a pool of 1000 indigenous LAB isolates.

Bacteriocin-like inhibitory substance screening

Food samples screened one thousand indigenous LAB isolates for BLIS (bacteriocin-like inhibitor substances) activity. From this pool of 1000 LAB, 280 isolates showed antagonistic activity by a different method in a solid medium. 46 isolates also showed antibacterial activity in broth, as indicated by pH-neutralized cell-free supernates. Eleven of these broth producers lost their activity after protease treatment, confirming the bacteriocin nature of the inhibitory substance.

Identification of Baccteriocin-producing LAB

The identification of bacteriocin-producing LAB isolates C27, C130 and C236 were carried out by API 20 STREP, and the strain was identified as Lactococcus lactis (Table 3). The identification of strain C130 was confirmed using the 16s rRNA targeted PCR method. Approximately 1500 bp PCR amplification product indicated the presence of L. lactis; Figure 3 shows PCR-based identification.

Antimicrobial spectrum of bacteriocins

The CFS of C27, C130, and C236 were tested against different food spoilage and pathogenic bacteria (Table 3). None of the bacteriocins inhibited gram-negative bacteria, and all of them were active against gram-positive bacteria, including Staphylococcus aureus. C130 showed a fair spectrum against B. cereus (Table 4).

Thermal and pH stability of bacteriocins

Bacteriocin of strain C130 was found to be thermostable even after 10 minutes of treatment at 100ºC (Table 2). All three bacteriocins reported in this study were found to be most active at acidic pH when checked in a pH range of 2 to 9 (Table 2).

Determination of strain L. lactic C130 growth for maximum bacteriocin production

Bacteriocin production during the growth of C130 was monitored for 48 hours. The culture started to produce bacteriocin during late logarithmic growth and reached a peak in the stationary phase.

Table 4: Bacteriocin production of strain C-130 with time-course experiment versus optical density with bacteriocin activity (AU/ml).

Time (hours)	Bacteriocin AU/ml	Optical density
0	0	0.05
2	0	0.0729
3	0	0.128
4	0	0.21
5	0	0.3075
6	0	0.4075
7	0	0.5117
8	100	0.62
9	100	0.8114
10	100	0.9498
11	200	1.2374
12	200	1.6257
13	400	1.6258
14	400	1.6258
24	800	1.626
32	1600	1.627
48	800	1.6255

Discussion

In this study, we reported the presence and activity of lactic acid bacteria in fresh vegetables, raw milk, and fermented dairy products with high frequency. These LAB isolates, if characterized, may serve as good starters for industrial lactic acid fermentation processes. 28% of these LAB isolates exhibited inhibitory activity in a solid medium as judged by a deferred antagonistic assay.

Several other workers had also reported bacteriocin-like inhibitory activity producing LAB from various food sources[24,25]. This inhibitory activity in a solid medium may be due to the combined activity of bacteriocins and other fermentation end products of LAB, such as organic acids, diacetyl, aldehyde, and hydrogen peroxide[26]. 5% of LAB produced inhibitory activity in broth and agar, as shown by acid-neutralized CFS activity. Inhibitory substance production in broth depends upon the density and growth phase of the producer[27]. Many reports on bacteriocins are produced only in solid media[28-30].

Protease sensitivity is a key criterion for establishing the bacteriocin nature of inhibitory substances[31]. About 1% of our indigenous LAB isolates were found to lose their inhibitory activity after protease treatment, establishing the confirmation of its bacteriocin nature. Another important characteristic of LAB bacteriocin is their narrow antibacterial spectrum, usually active against Gram-positive bacteria[32]. Results of this study of the antibacterial spectrum also confirm the finding. Three LAB isolates showed activity against various food spoilage and foodborne bacteria, including Staphylococcus aureus. These three LAB isolates were labeled Lactococcus lactis by API 20S (low discrimination).

Molecular characterization of L. lactis C130 was performed by PCR using special primers for 16Sr RNA amplification. The amplified product of 1500 bp indicates the presence of the L. lactis genome in C130, confirming API identity.

Bacteriocin of L. lactis C130 was found to be thermostable and retained activity after ten minutes at 100°C. The CFS of all three strains retained bacteriocin activity after storage at 4°C for 30 days (results not shown). There are other reports of heat-stable bacteriocins of LAB in literature[33,34]. Heat stability of bacteriocin is a good feature during food preservation[35]. Bacteriocins of L. lactis C130, C27, and C236 were stable and active at acidic pH. Other researchers[34] also report this result in increased bacteriocin activity during acidic pH. Acidic pH stability of bacteriocins makes them useful preservatives in fermented foods[35]. In this study, the growth of heat-stable bacteriocin producing L. lactis C130 was measured spectrophotometrically for maximum bacteriocin production in MRS broth at 30°C without pH control. Maximum bacteriocin activity was found during the late exponential and stationary phase. Many reports in the literature describe bacteriocin production in LAB as an inducible characteristic that depends upon the cell density of culture as well as physical and nutritional factors[34,35].

The major limitation of the study was that we were unable to purify the crude BLIS. Hence a more efficient way of work than the existing recommended protocols to purify the crude BLIS must be designed.

Conclusion

In this study, we report the screening of food and dairy samples for isolation of indigenous LAB that was bacteriocin producers. After phenotypic and molecular characterization, L. lactis C 130 was found to be a bacteriocin producer. It produced a thermostable bacteriocin with a fair antibacterial spectrum against various food spoilage and pathogenic gram-positive bacteria. The bacteriocin was produced during the late exponential growth of L. lactis C130, reaching a peak during the stationary phase. Further work is needed to gain more information about bacteriocin production and applications.

Conflicts of Interest

The authors have declared that no competing interests exist.

Acknowledgement

The authors thank the Department of Microbiology, the University of Karachi, for providing research facilities.

Funding

There was no funding, and the Department of Microbiology, the University of Karachi, supported using the existing facilities.

References

1. Zhang T, Zhang Y, Li L, Jiang X, Chen Z, Zhao F, Yi Y. Biosynthesis and Production of Class II Bacteriocins of Food-Associated Lactic Acid Bacteria. Ferment. 2022;8(5):217.
2. Maldonado-Barragán A, West SA. The cost and benefit of quorum sensing-controlled bacteriocin production in Lactobacillus plantarum. J Evol Biol. 2020;33(1):101-111.
3. Yi Y, Li P, Zhao F, Zhang T, Shan Y, Wang X, Liu B, Chen Y, Zhao X, Lü X. Current status and potentiality of class II bacteriocins from lactic acid bacteria: Structure, mode of action and applications in the food industry. Trends Food Sci Technol. 2022.
4. Verma DK, Thakur M, Singh S, Tripathy S, Gupta AK, Baranwal D, Patel AR, Shah N, Utama GL, Niamah AK, Chávez-González ML. Bacteriocins as antimicrobial and preservative agents in food: Biosynthesis, separation and application. Food Biosci. 2022;46:101594.
5. Messens W, De Vuyst L. Inhibitory substances produced by Lactobacilli isolated from sourdoughs—a review. Int J Food Microbiol. 2002;72(1-2):31-43.
6. Piard JC, Desmazeaud M. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. Le lait. 1992;72(2):113-142.
7. Meng S, Xu H, Wang F. Research advances of antimicrobial peptides and applications in food industry and agriculture. Curr Protein Pept Sci. 2010;11(4):264-273.

8. Perez RH, Zendo T, Sonomoto K. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microb Cell Factories. 2014;13(1): Article S3.
9. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait?. Appl Environ Microbiol. 2012;78(1):1-6.
10. Bharti VA, Mehta AR, Singh SI, Jain NE, Ahirwal LA, Mehta SU. Bacteriocin: a novel approach for preservation of food. Int. J. Pharm. Pharm. Sci. 2015;7(9):20-29.
11. Hammami R, Zouhir A, Le Lay C, Ben Hamida J, Fliss I. BACTIBASE second release: a database and tool platform for bacteriocin characterization. Bmc Microbiology. 2010;10(1):1-5.
12. Rea MC, Ross RP, Cotter PD, Hill C. Classification of bacteriocins from Gram-positive bacteria. InProkaryotic antimicrobial peptides 2011 (pp. 29-53). Springer, New York, NY.
13. Aljewicz M, Cichosz G. Influence of probiotic (Lactobacillus acidophilus NCFM, L. paracasei LPC37, and L. rhamnosus HN001) strains on starter cultures and secondary microflora in Swiss-and Dutch-type cheeses. J Food Process Preserv. 2017;41(6):e13253.
14. Sobrino-López A, Martín-Belloso O. Use of nisin and other bacteriocins for preservation of dairy products. Int Dairy J. 2008;18(4):329-343.
15. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ. Application of natural antimicrobials for food preservation. J Agric Food Chem. 2009;57(14):5987-6000.
16. Sidooski T, Brandelli A, Bertoli SL, Souza CK, Carvalho LF. Physical and nutritional conditions for optimized production of bacteriocins by lactic acid bacteria–A review. Critical reviews in food science and nutrition. 2019;59(17):2839-2849.
17. De Vuyst L, Callewaert R, Crabbé K. Primary metabolite kinetics of bacteriocin biosynthesis by Lactobacillus amylovorus and evidence for stimulation of bacteriocin production under unfavourable growth conditions. Microbiol. 1996;142(4):817-827.
18. Manzoor A, Ul-Haq I, Baig S, Qazi JI, Seratlic S. Efficacy of locally isolated lactic acid bacteria against antibiotic-resistant uropathogens. Jundishapur j microbiol. 2016;9(1).
19. Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, Gänzle M, Kort R, Pasin G, Pihlanto A, Smid EJ. Health benefits of fermented foods: microbiota and beyond. Curr Opin Biotechnol. 2017;44:94-102.
20. De Man JC, Rogosa D, Sharpe ME. A medium for the cultivation of lactobacilli. J Appl Microbiol. 1960;23(1):130-135.
21. Hechard Y, Dherbomez M, Cenatiempo Y, Letellier F. Antagonism of lactic acid bacteria from goats' milk against pathogenic strains assessed by the 'sandwich method'. Lett Appl Microbiol. 1990;11(4):185-188.
22. Mayr-Harting A, Hedges AJ, Berkeley RC. Chapter VII methods for studying bacteriocins. InMethods in microbiology 1972 Jan 1 (Vol. 7, pp. 315-422). Academic Press.
23. Joerger MC, Klaenhammer TR. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by Lactobacillus helveticus 481. J bacteriol. 1986;167(2):439-446.
24. Todorov SD. What bacteriocinogenic lactic acid bacteria do in the milk?. InRaw Milk 2019 pp. 149-174. Academic Press.
25. Qiao X, Du R, Wang Y, Han Y, Zhou Z. Isolation, characterisation and fermentation optimisation of bacteriocin-producing Enterococcus faecium. Waste Biomass Valorization. 2020;11(7):3173-3181.
26. Knorr D. Technology aspects related to microorganisms in functional foods. Trends Food Sci Technol. 1998;9(8-9):295-306.
27. Prazdnova EV, Gorovtsov AV, Vasilchenko NG, Kulikov MP, Statsenko VN, Bogdanova AA, Refeld AG, Brislavskiy YA, Chistyakov VA, Chikindas ML. Quorum-Sensing Inhibition by Gram-Positive Bacteria. Microorganisms. 2022;10(2):350.
28. Fricourt BV, Barefoot SF, Testin RF, Hayasaka SS. Detection and activity of plantaricin F an antibacterial substance from Lactobacillus plantarum BF001 isolated from processed channel catfish. J Food Prot. 1994;57(8):698-702.
29. Yang E, Fan L, Yan J, Jiang Y, Doucette C, Fillmore S, Walker B. Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. Amb Express. 2018;8(1):1-4.
30. Azevedo PO, Molinari F, Oliveira RP. Importance of the agar-media in the evaluation of bacteriocin activity against the same test-microorganisms. Braz J Pharm Sci. 2018;54.
31. Xiang YZ, Wu G, Yang LY, Yang XJ, Zhang YM, Lin LB, Deng XY, Zhang QL. Antibacterial effect of bacteriocin XJS01 and its application as antibiofilm agents to treat multidrug-resistant Staphylococcus aureus infection. Int J Biol Macromol. 2022;196:13-22.
32. Gradisteanu Pircalabioru G, Popa LI, Marutescu L, Gheorghe I, Popa M, Czobor Barbu I, Cristescu R,

Chifiriuc MC. Bacteriocins in the era of antibiotic resistance: rising to the challenge. Pharmaceutics. 2021;13(2):196.

33. Sharma S, Garg AP, Singh G. Optimization of fermentation conditions for bacteriocin production by Lactococcus lactis CCSULAC1 on modified MRS medium. Int J Dairy Sci. 2010;5(1):1-9.
34. Imade EE, Omonigho SE, Babalola OO, Enagbonma BJ. Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria. Ann microbiol. 2021;71(1):1-4.
35. O'sullivan DJ, Lee JH. U.S. Patent No. 7,960,505. Washington, DC: U.S. Patent and Trademark Office. 2011.