

ISOLATION AND IDENTIFICATION OF BACTERIOCIN PRODUCING MICROBES USING BIOCHEMICAL AND MOLECULAR TOOLS AND ANALYSIS OF ITS BIOPRESERVATION POTENTIAL

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ABSTRACT

Objective: Food safety is a matter of utmost importance in developing countries as well as in developed countries, so keeping this in mind this research work deals with the identification and characterization of bacteriocin producing microbes by using biochemical and molecular characterization. This study has also covered the biopreservation potential of bacteriocin produced by these microbes against sapodilla, tomato and banana as well.

Methods: For the purpose of sample collection and isolation, samples of milk, curd and gangajal water were taken and bacteriocin producing microbes were isolated by using serial dilution method. Screening of bacteriocin producing microbe was done by antibacterial sensitivity test using agar well diffusion method against *Bacillus amyloliquefaciens*, *Escherichia Coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by determining their zone of inhibition. Biochemical characterization was done by using different tests, such as, catalase test, mannitol test, citrate test, gelatin test, maltose test, indole test, urease test, lactose test etc. Molecular characterization was done by using 16S rRNA gene sequencing. Preservative action of bacteriocin was observed on fruits that comprise sapodilla, tomato and banana by spraying bacteriocin on them and analyzing their activities shows for at least 10 days.

Results: Microbes were found to be *Enterococcus faecalis* (Accession number KX011874) and *Bacillus cereus* (Accession number KX011875). Periodic observatory studies reflect that using bacteriocin, banana can be preserved for nearly 6-7 days while sapodilla for 8-9 days and tomato for 9-10 days.

Conclusion: From present study we would like to conclude that bacteriocins produced by microbes which is found in milk or curd can also be used as biopreservatives for these defined fruits that is sapodilla, tomato and banana.

Keywords: Bacteriocin, Biopreservation, 16S rRNA analysis.

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INTRODUCTION

Many chemicals are being used for inactivation of foodborne pathogens so as to preserve food products for a longer duration [1]. Chemical preservatives have some of the undesirable side effects such as alteration in the constituents, nutritional, and organoleptic properties of the food and toxic effect on human health [1]. As far as the natural preservatives are concerned, they have shown a better alternative. Bacteriocin which is a natural product is isolated from milk, curd, cheese etc. can be used as a biopreservative. Bacteriocins are proteinaceous toxin produced by bacteria to inhibit the growth of similar or closely related bacteria [2]. It was first discovered by Gratia in 1925, when he was searching for ways to kill bacteria [3,4]. Bacteriocin is used as a preservative in food due to its heat stability, wider pH tolerance, and its proteolytic activity [5]. Due to thermostability and pH tolerance, it can withstand heat and acidity/alkalinity of food during storage condition [5]. Bacteriocins are commonly divided into three or four groups (Klaenhammer, 1993; Nes *et al.*, 1996). Nisin was discovered in 1928 (Hurst, 1967) and subtilin, a nisin analog differing by 12 amino acid residues was discovered in 1948 (Hansen, 1993). Both belong to Class I, termed lantibiotics [6]. Bacteriocins of lactic acid bacteria are considered to be safe biopreservatives, since they are assumed to be degraded by the proteases in the gastrointestinal tract [6]. The aim of this study was to characterize the bacteriocin producing microbes and also to observe the biopreservation potential of bacteriocin producing microbes. Reported studies on Bacteriocin isolated from different sources are shown in the Table 1 and reported studies on preservative action of Bacteriocin are shown in Table 2. In the mean while this study also tried to find whether bacteriocin producing microbe is present in gangajal water or not.

METHODS

Source of sample collection

Samples of milk and curd were collected from a family in Sohna, Haryana, India. The sample of gangajal was collected from Haridwar, Uttarakhand, India.

Bacterial strain

Bacterial strain of *Bacillus amyloliquefaciens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* was obtained from Helix BioGenesis Pvt. Ltd., Noida, Uttar Pradesh, India. They were subcultured freshly in Luria broth (LB media) and used further for research work.

Isolation

Samples were simultaneously inoculated to the LB and incubated at 37°C on continuous shaking at 150 rpm. After incubation, test tube was observed for the growth of bacteria. Serial dilution was done to know the bioburden of sample. Different colonies were isolated from LB agar plate after serial dilution from different samples. Isolated colonies were streaked onto freshly prepared LB agar. Pure cultures of these isolates were obtained by picking up the colonies. Glycerol stock was prepared to maintain the culture of isolates at -80°C.

Screening of bacteriocin producing microbes

Antibacterial sensitivity test (agar well diffusion method)

Antibacterial feature of bacteriocin against selected microorganisms (*B. amyloliquefaciens*, *E. coli*, *S. aureus*, and *P. aeruginosa*) were investigated by agar well-diffusion method. LB agar media were prepared and autoclaved at 121°C for 15 minutes at 15 lbs

and poured in sterile Petri plate up to a uniform thickness of approximately 10-15 minutes, and the agar was allowed to set at ambient temperature. This method is suitable for the organism to grow rapidly overnight at 35-37°C. The wells were made in medium after inoculation with the microorganism. 200 ml of inoculums were spread over LB agar plate using sterile spreader after few minutes 4 wells were made in each Petri plate and loaded with 100 ml of isolates. Plates were incubated at 37°C for 24 hrs. Antibacterial activity was observed by measuring its zone of inhibition. The experiments were done in triplicate.

Morphological and biochemical characterization of the bacteriocin producing isolates

Isolated colonies were characterized morphologically and biochemical test (Indole test, methyl red test, voges-proskauer test, citrate test, urease test, mannitol test, catalase test, starch hydrolysis, growth at 5% NaCl, growth at 10% NaCl, lactose test, maltose test, dextrose test, growth at 4°C and gelatin test) were carried out to authenticate their identity by Cappuccino and Sherman, 1989.

Molecular characterization of bacteriocin producing microbes

DNA was isolated by following standard methods (Sambrook *et al.* 1989), as modified by Baker *et al.*, 1994 and 16S rDNA was amplified using the 16S rDNA primers, F (5'-AGAGTTTGATCMTGGCTCAG-3') and R (5'-TACGGYTACCTTGTACGACTT-3'). Polymerase chain reaction (PCR) was performed with 25 µl reaction mix by taking 2.5 µl of 10X Taq buffer, 15 mM MgCl₂, 1 µl of dNTPs concentration, 1 µl of reverse primer and 1 µl of forward primer, 15.30 µl of water, 4 µl of DNA sample, and 0.2 µl of Taq polymerase. PCR was subjected to 30 cycles (denaturation, 30 seconds at 95°C; annealing, 30 seconds at 55°C; extension, 1 minute at 72°C) and 1 final extension cycle at 72°C for 10 minutes. PCR products were analyzed by agarose gel electrophoresis, which was performed using 1% agarose gel and EtBr was used to visualize DNA. 8-10 µl of DNA sample was loaded in the wells and it was allowed to run at 70-80 V and analyzed under ultraviolet light.

Biopreservative action of bacteriocin

Biopreservative action of bacteriocin was observed on sapodilla, banana, and tomato. Bacteriocin isolates were sprayed on sapodilla, banana, and tomato and they were observed daily for their biopreservative action. The appearance of bacteriocin treated food products was compared with the control sample.

Table 1: Reported studies on Bacteriocin isolated from different sources

Sl No.	Reported studies
1	Bacteriocin production by a new isolate of <i>Lactobacillus rhamnosus</i> GPI under different culture conditions [7]
2	Purification and Characterization of a Bacteriocin Produced by <i>Lactobacillus lactis</i> Isolated from Marine Environment [8]
3	Production of bacteriocin like substances by lactic acid bacteria isolated from regional ovine cheese [9]
4	Isolation and characterization of nisin producing <i>Lactococcus lactis</i> subsp. <i>lactis</i> from bean-sprouts [10]
5	Characteristics and genetic determinants of bacteriocin activities produced by <i>Carnobacterium piscicola</i> isolated from cheese [11]
6	Purification and characterization of bacteriocin produced by <i>Lactobacillus plantarum</i> isolated from cow milk [12]
7	Characterization of bacteriocin produced by <i>Lactobacillus plantarum</i> and <i>Lactobacillus brevis</i> [13]
8	Purification and characterization of bacteriocin Produced by <i>Lactobacillus brevis</i> isolated from Dhulliachar: a traditional food product of North East India [14]

RESULTS

Isolation

After inoculation of samples in LB broth, turbidity in LB broth was taken as a primary indicator for microbial growth. Nine morphologically different isolates were obtained from gangajal water, curd, and milk, whereas isolates 1 and 2 obtained from gangajal water, isolates 3, 4, 5, and 6 from curd while isolates 7, 8, and 9 from milk.

Antibacterial sensitivity test

For identifying antibacterial sensitivity test, antibacterial activity was observed against *B. amyloliquefaciens*, *E. coli*, *S. aureus*, and *P. aeruginosa* by determining their zone of inhibition. Hence, in this study, isolates 2, 5 and 6 has not shown any antibacterial activity against *B. amyloliquefaciens*, whereas isolates 1, 3, 4, 7, 8, and 9 has shown antibacterial activity against the same. As far as *E. coli* was concerned isolates 1, 3, and 6 has not shown any inhibition, whereas *E. coli* found to be inhibited by isolates 2, 4, 5, 7, 8, and 9 as given in Table 1. Isolate 4 and 9 were selected for 16S rRNA gene sequence analysis as these two samples were showing better result for well diffusion method compared to other isolates. Antibacterial activity of bacteriocin in isolates 3 and 4 is shown in Fig. 1 and zone of inhibition for antibacterial activity is shown in Table 3.

Morphological and biochemical characterization

From this study, nine different isolates were morphologically characterized by Gram-staining and it has shown in the presence of Gram-positive bacteria with rod shape in its isolates 1 and 4 while cocci shape in isolates 2, 5, and 9, whereas Gram-negative bacteria with rod shape found to be present in isolates 3, 6, 7, and 8. Biochemical characterization has revealed the presence of methyl red in isolates 1, 3, 4, 7, and 9, whereas absent in isolates 2, 5, and 8. Voges-proskauer was present in isolates 1, 3, 4, 5, 7, and 9 while absent in isolates 2, 6, and 8. Indole was found to be absent in all

Table 2: Reported studies on preservative action of Bacteriocin

Sl No.	Reported studies
1	Biochemical and Genetic Characterization of Enterocin P, a Novel sec-Dependent Bacteriocin from <i>Enterococcus faecium</i> P13 with a Broad Antimicrobial Spectrum [15]
2	The use of the bacteriocin, nisin, as a preservative in ricottatype cheeses to control the foodborne pathogen <i>Listeria monocytogenes</i> [16]
3	Evidence for a bacteriocin like substance produced by a new strain of <i>Streptococcus</i> sp., inhibitory to gram positive Food-borne pathogens [17]
4	Effective use of nisin to control lactic acid bacterial spoilage in vacuum-packed Bologna-type sausage [18]
5	Applications of bacteriocins in food, livestock health and medicine [19]
6	Application of bacteriocins in vegetable food biopreservation [20]
7	Isolation of a <i>Lactobacillus salivarius</i> Strain and Purification of its Bacteriocin, which is inhibitory to <i>Campylobacter jejuni</i> in the Chicken Gastrointestinal System [21]
8	Application of Lactic Acid Bacteria and their bacteriocins for food Biopreservation [22]
9	Application of the broadspectrum bacteriocin enterocin AS-48 to inhibit <i>Bacillus coagulans</i> in canned fruit and vegetable foods [23]
10	Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods [24]
11	Class IIa bacteriocins: biosynthesis, structure and activity [25]
12	Antibiotic and Bacteriocin Sensitivity of <i>Listeria monocytogenes</i> strains isolated from different foods [26]

isolates. Citrate was found be present only in isolate 2. Urease was found to be present in isolates 6 and 8 and absent in other isolates. Mannitol was found to be absent in all isolates. Catalase was found to be present in isolates 2, 3, 4, 5, 6, and 8 while absent in isolates 1, 7, and 9. Starch hydrolysis was found in isolates 4 and 5. Growth at 5% NaCl was found in isolates 1, 3, 4, and 5. Growth at 10% NaCl was not found in any isolates. Gelatin was found to be absent in all isolates. Lactose was found to be present in isolate 8 while absent in other isolates. Maltose was found to be present in isolates 1, 3, 7, and 9 while absent in isolates 2, 4, 5, 6, and 8. Dextrose was found to be present in isolates 1, 2, 3, 5, 6, 7, 8, and 9 while absent in isolate 4. Growth at 4°C was not found in any isolates. Biochemical characterization for nine isolates is shown in Table 4.

Molecular characterization

Using 16S rRNA gene sequence analysis Gram-positive bacterium isolate 4 and 9 were identified, their sequences have been submitted to the National Center for Biotechnology Information GeneBank and their accession number has been achieved. Isolate 4 found to be *Bacillus cereus* (Accession number KX011875) and isolate 9 found to be *Enterococcus faecalis* (Accession number KX011874).

Using phylogeny.fr (http://www.phylogeny.fr/simple_phylogeny.cgi), phylogenetic tree was formed as shown in Figs. 2 and 3.

Biopreservative action of bacteriocin

Biopreservation activity for isolates 4 and 9 was done on fruit samples sapodilla, banana, and tomato. Fruit samples were sprayed with bacteriocin, and these were checked periodically for 10 days. Test samples were regularly compared with control. These periodic observatory studies reflect that using bacteriocin, banana can be preserved for nearly 6-7 days while sapodilla for 8-9 days and tomato for 9-10 days. Biopreservative action of bacteriocin for different fruits is shown in Table 5.

DISCUSSION

Our result suggested that bacteriocins producing *E. faecalis* (Accession number KX011874) and *Bacillus cereus* (Accession number KX011875) naturally occur and survive in milk and curd, respectively. The present study has shown the significant applications of bacteriocin (isolated from *Bacillus cereus* and *E. faecalis*) as a biopreservative by applying on tomato, banana, and sapodilla as well. This research has clearly shown the comparative analysis between the control and test sample through observatory studies. An earlier study has been done on strains producing bacteriocins such as *Enterococcus mundtii* (Bennik et al., 1998), *Lactobacillus lactis* subsp. cremoris R (Yildirim and Johnson, 1998), *Lactobacillus bavaricus* (bavA) (Larsen et al., 1993), *Carnobacterium piscicola* CP5 (Herbin et al., 1997), etc. Apart from

Table 3: Antibacterial activity of bacteriocin

Isolates	<i>Bacillus amyloliquefaciens</i> (Zone of inhibition)	<i>Escherichia coli</i> (Zone of inhibition)	<i>Staphylococcus aureus</i> (Zone of inhibition)	<i>Pseudomonas aeruginosa</i> (Zone of inhibition)
1	0.013 m	0	0	0
2	0	0.015 m	0	0
3	0.032 m	0	0.015 m	0
4	0.021 m	0.015 m	0.015 m	0.031 m
5	0	0.013 m	0.018m	0
6	0	0	0	0.018 m
7	0.014 m	0.013 m	0	0
8	0.015 m	0.013 m	0	0.012 m
9	0.017 m	0.018 m	0	0.016 m

Table 4: Biochemical characterization

Tests	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9
Indole test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
MR test	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive
VP test	Positive	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Positive
Citrate test	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Urease test	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative
Mannitol test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Catalase test	Negative	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative
Starch hydrolysis	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative
Growth at 5% NaCl	Positive	Negative	Positive	Positive	Positive	Negative	Negative	Negative	Negative
Growth at 10% NaCl	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Lactose test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Maltose test	Positive	Negative	Positive	Negative	Negative	Negative	Positive	Negative	Positive
Dextrose test	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Growth at 4°C	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Gelatin test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 5: Biopreservative action of bacteriocin for different fruits

Days	Banana	Sapodilla	Tomato	Control
1 st day	Appeared fresh	Appeared fresh	Appeared fresh	Appeared fresh
4 th day	Appeared fresh	Appeared fresh	Appeared fresh	Spoiled up to some extent
6 th day	Spoiled up to some extent	Appeared fresh	Appeared fresh	Started to spoil rapidly
8 th day	Started to spoil rapidly	Spoiled up to some extent	Spoiled up to some extent	Spoiled completely

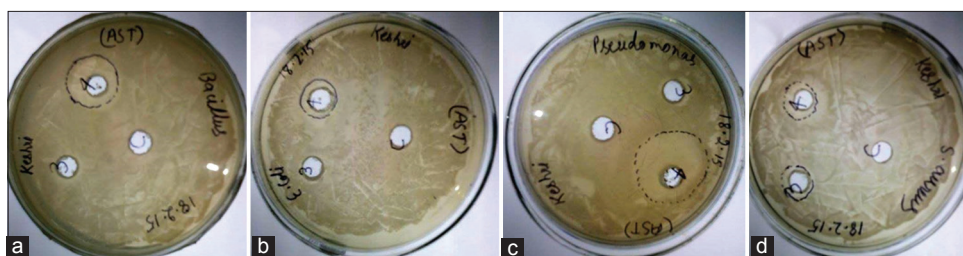


Fig. 1: Antibacterial activity of bacteriocin in isolates 3 and 4 against (a) *Bacillus amyloliquefaciens*, (b) *Escherichia coli*, (c) *Staphylococcus aureus*, and (d) *Pseudomonas aeruginosa*

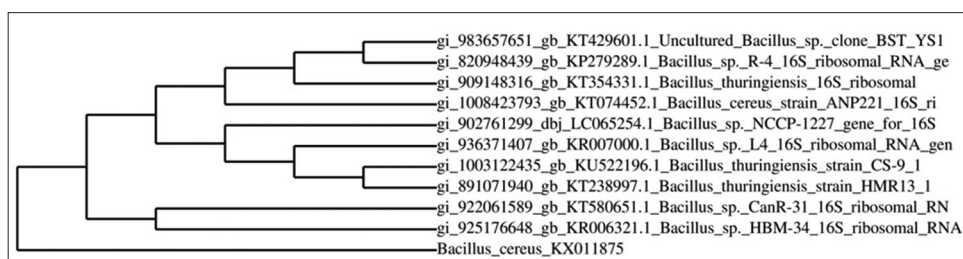


Fig. 2: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence of *Bacillus cereus* KX011875

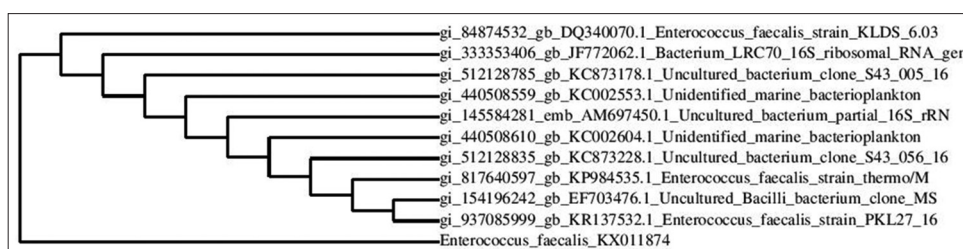


Fig. 3: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence of *Enterococcus faecalis* KX011874

studying on isolates from milk and curd, this paper also tried to study on isolates from gangajal water as well, to find whether the bacteriocin producing microbes are present in gangajal or not, although gangajal was not having the bacteriocin producing microbe, so far, no such study on gangajal water has been done in the past. This study would surely provide a preliminary foundation for further studies in exploring the other probable outcomes and applications of bacteriocin.

CONCLUSION

This research work has properly characterized and identified the bacteriocin producing microbes on the basis of biochemical and molecular characterization. It has also been shown that bacteriocin treated food products can stay fresh for a longer period. So, it demonstrates the effectiveness of the preservative action of bacteriocin. Thus, bacteriocin can be suggested to be used as a safe, natural, and effective biopreservative.

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REFERENCES

1. Bali V, Panesar PS, Bera MB. Isolation, screening and evaluation of antimicrobial activity of potential bacteriocin producing lactic acid bacteria isolate. *Microbiol J* 2011;1(3):113-9.
2. Deshmukh PV, Thorat PR. Bacteriocins: A new trend in antimicrobial food packaging. *Int J Adv Res Eng Appl Sci* 2013;2(1):1-2.

3. Gratia A. A remarkable example of antagonism between two strains of *E. coli*. *Compt Rend Soc Biol* 1925;93:1040-2.
4. Gratia JP, André Gratia: A forerunner in microbial and viral genetics. *Genetics* 2000;156(2):471-6.
5. Gautam N, Sharma N. Bacteriocin: Safest approach to preserve food products. *Indian J Microbiol* 2009;49(3):204-11.
6. Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: Safe, natural antimicrobials for food preservation. *Int J Food Microbiol* 2001;71(1):1-20.
7. Sarika AR, Lipton AP, Aishwarya MS. Bacteriocin production by a new isolate of *Lactobacillus rhamnosus* GPI under different culture conditions. *Adv J Food Sci Technol* 2010;2(5):291-7.
8. Rajaram G, Manivasagam P, Thilagavathi B, Saravanakumar A. Purification and characterization of a bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. *Adv J Food Sci Technol* 2010;2(2):138-44.
9. Nespolo CR, Brandelli A. Production of bacteriocin-like substances by lactic acid bacteria isolated from regional ovine cheese. *Braz J Microbiol* 2010;41(4):1009-18.
10. Cai Y, Ng LK, Farber JM. Isolation and characterization of nisin-producing *Lactococcus lactis* subsp. *lactis* from bean-sprouts. *J Appl Microbiol* 1997;83(4):499-507.
11. Herbin S, Mathieu F, Brulé F, Branlant C, Lefebvre G, Lebrihi A. Characteristics and genetic determinants of bacteriocin activities produced by *Carnobacterium piscicola* CP5 isolated from cheese. *Curr Microbiol* 1997;35(6):319-26.
12. Sankar NR, Priyanka VD, Reddy PS, Rajanikanth P, Kumar VK, Indira M. Purification and characterization of bacteriocin produced by *Lactobacillus plantarum* isolated from cow milk. *Int J Microbiol Res* 2012;3(2):133-7.
13. Ogunbanwo ST, Sanni AI, and Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Afr J Biotechnol* 2003;2(8):219-27.

14. Gautam N, Sharma N, Ahlawat OP. Purification and characterization of bacteriocin produced by *Lactobacillus brevis* UN Isolated from Dhulliachar: A traditional food product of North East India. *Indian J Microbiol* 2014;54(2):185-9.
15. Cintas LM, Casaus P, Håvarstein LS, Hernández PE, Nes IF. Biochemical and genetic characterization of enterocin P, a novel sec-dependent bacteriocin from *Enterococcus faecium* P13 with a broad antimicrobial spectrum. *Appl Environ Microbiol* 1997;63(11):4321-30.
16. Davies EA, Bevis HE, Delves-Broughton J. The use of the bacteriocin, nisin, as a preservative in ricotta-type cheeses to control the food-borne pathogen *Listeria monocytogenes*. *Lett Appl Microbiol* 1997;24(5):343-6.
17. Gomez S, Cosson C, Deschamps AM. Evidence for a bacteriocin-like substance produced by a new strain of *Streptococcus* sp. inhibitory to gram-positive food-borne pathogens. *Res Microbiol* 1997;148(9):757-66.
18. Davies EA, Milne CF, Bevis HE, Potter RW, Harris JM, Williams GC, et al. Effective use of nisin to control lactic acid bacterial spoilage in vacuum-packed bologna-type sausage. *J Food Prot* 1999;62(9):1004-10.
19. Bemena LD, Mohamed LA, Fernandes AM, Lee BH. Applications of bacteriocins in food, livestock health and medicine. *Int J Curr Microbiol Appl Sci* 2014;3(12):924-49
20. Settanni L, Corsetti A. Application of bacteriocins in vegetable food biopreservation. *Int J Food Microbiol* 2008;121(2):123-38.
21. Stern NJ, Svetoch EA, Eruslanov BV, Pereygin VV, Mitsevich EV, Mitsevich IP, et al. Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrob Agents Chemother* 2006;50(9):3111-6.
22. Gálvez A, López RL, Pulido RP, Burgos MJ. Application of lactic acid bacteria and their bacteriocins for food biopreservation. *Food Biopreservation Springer Briefs in Food, Health, and Nutrition*. New York: Springer-Verlag; 2014. p. 15-22.
23. Lucas R, Grande MA, Abriouel H, Maqueda M, Ben Omar N, Valdivia E, et al. Application of the broad-spectrum bacteriocin enterocin AS-48 to inhibit *Bacillus coagulans* in canned fruit and vegetable foods. *Food Chem Toxicol* 2006;44(10):1774-81.
24. Schillinger U, Geisen R, Holzapfel WH. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends Food Sci Technol* 1996;7:158-64.
25. Ennahar S, Sashihara T, Sonomoto K, Ishizaki A. Class Iia bacteriocins: Biosynthesis, structure and activity. *FEMS Microbiol Rev* 2000;24(1):85-106.
26. Kocan EG, Cosansu S, Ayhan K, Juneja VK, Materon L. Antibiotic and bacteriocin sensitivity of *Listeria monocytogenes* strains isolated from different foods. *Food Nutr Sci* 2012;3:363-8.