

Purification of bacteriocin BacPC from *Pediococcus pentosaceus* sp. suitable for foods preservation

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Abstract: Bacteriocins were metabolized products of food – grad, microorganisms and some of which were used as food preservative to improve the food safety. Bacteriocin producing *Pediococcus pentosaceus* (BacPC) was protein in nature with antimicrobial effects on some clinically important food borne pathogens. This strain was isolated from the milk products samples and they were cultivated on MRS agar and identified using biochemical methods. The purification technologies, the activity determination, and the characteristics of the bacteriocin were also explained. The results suggested that the protein bands were observed with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as a single band with a molecular mass 19 KDa (BacPC). The mode of action of the partial purified Bacteriocin was identified as bactericidal against *Staphylococcus aureus*. The minimal inhibitory concentration (MIC) of bacteriocin (BacPC) for the indicator organism *Staphylococcus aureus* was 6 mg.ml⁻¹. Results presented here support the idea that the bacteriocin may propose some industrial advantage that renders it as a good natural food biopreservative candidate.

Keywords: *Pediococcus pentosaceus* sp., *Staphylococcus aureus*, bacteriocins, purification, MIC.

1. Introduction

Bacteriocins, are large and functionally diverse family of antimicrobials found in all major lineages of bacteria. Recent studies reveal that these proteinaceous toxins play a significant role in mediating competitive dynamics between bacterial strains and closely related species (1). Among natural biological antagonists, Lactic Acid Bacteria group (LAB) which are mainly divided into four genera: *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*, they have several potential applications and are widely used for the production of fermented foods and are also part of intestinal micro-flora (2). These bacteria have a long history of use in foods, they produce some antagonistic compounds able to control pathogenic bacteria and undesirable spoilage micro flora, in particular using LAB to control mold growth could be an interesting alternative to physical and chemical methods because these bacteria have been reported to have strong antimicrobial properties (3).

Several strategies for isolation and purification of bacteriocins from complex cultivation broths to final products were described. Biotechnological procedures including salting out, solvent extraction, ultrafiltration, adsorption-desorption, ion-exchange, and size exclusion chromatography are among the most usual methods (4). The presence of hydrophobic regions in bacteriocin molecules is essential for their activity against sensitive bacteria. Inactivation of micro-organisms by bacteriocins depends on the hydrophobic interaction between cells and bacteriocin molecules (5). The amphiphilic properties of bacteriocins have been used to separate these peptides at the interface of immiscible liquids (6). Peptide structure-function studies of bacteriocins and bacterial genetic advances will help to understand the molecular basis of their specificity and mode of action (7, 5). The objective of this study was to purify the bacteriocin (BacPC) of *Pc. pentosaceus* sp. by molecular

techniques, determination of the CIM and CMB for *Staphylococcus aureus*, and study the possibility of using this LAB or its bacteriocin as a preservative in foods.

2. Materials and Methods

2.1. Strains and culture conditions

One hundred and forty one (141) LAB isolates from milks products, and thirteen indicator organisms were used in this study. All bacterial cultures were kept at -80°C in 40% glycerol until needed. Lactic acid bacteria strains were grown in De Man, Rogosa and Sharpe (MRS) (Merck, Germany) at 37°C for 2-3 days (pH 6.5) under aerobiosis and anaerobiosis conditions, non-LAB strains were grown in nutrient broth at 37°C in aerobic conditions (8, 9).

Bacteriocin positive colonies with a large diameter of zone of inhibition were identified according to Bergey's manual of determinative bacteriology (1994) and selected for the further bacteriocin production and characterization studies (9).

Pc.pentosaceus sp. was chosen for further experiments and characterization of their antimicrobial activity. Their selection was based on the display of high bacteriocin activity and their potential of inhibiting the growth of *S. aureus* using the agar spot method (9).

2.2. Production of bacteriocin-like substance

Pc.pentosaceus was inoculated in MRS broth and incubated at 37°C for 18 h. The cultures were centrifuged at $10.000 \times g$ for 10 min at 4°C . The supernatants were collected and sterilized by filtering through a $0.22\text{-}\mu\text{m}$ filter (Renner GMBH D-67125/Germany). The pH of the sterile supernatant was adjusted to 6.5-7 using 5 N NaOH or 5 N HCl, and 1 mg/mL of catalase was added to remove any hydrogen peroxide. The supernatants were incubated at 37°C for 2 h and then placed in a 60°C water bath for 10 min to inhibit any enzymes (10).

2.3. Physical and biochemical characterization of bacteriocin-like substance

2.3.1. The influence of heat, pH, and UV

The thermal stability of crude bacteriocin preparations was assessed by exposing the supernatant to different temperatures ranging from 0°C to 121°C (0°C , 30°C , 40°C , 50°C , 60°C , 70°C , 80°C , 90°C , 100°C and (121°C and 15 Lbs) for 15 minutes before being tested for their antimicrobial activity. The effect of pH on the activity of bacteriocin was tested by adjusting supernatant from 2 to 12 (at increment of one pH unit) with sterile 1 N NaOH or 1N HCl (11), after one hour of incubation at room temperature (25°C), the samples were tested for antimicrobial activity by the ODM. Similarly, sterile petri dishes containing aliquots of 10 mL crude bacteriocin preparations were exposed to the UV irradiation (Philips bulb, wavelength 340 nm, 220-240 V, 50 Hz,) situated at a distance of 30 cm (12, 13). Times of exposure to UV light ranged from 15 to 75 min. After each time interval, bacteriocin activity was estimated to UV light by ODM as previously stated together with unexposed bacteriocin-containing supernatants that served as the experimental controls.

2.3.2. Stability during storage

The method devised by (13) was used to study the stability of bacteriocin preparations during different storage conditions. The crude bacteriocin was stored at -20°C and $+4^{\circ}\text{C}$ for different interval of time (15, 30, 45, 60, and 90 days). Samples were taken from the stored material to determine the bacteriocin activity as previously mentioned. Bacteriocin-containing supernatants of producer strain that was not subjected to storage, and inoculated with the same indicator strain served as control.

2.3.3. Partial purification of bacteriocin

The cell free extract was collected by spun the tube at 15,000 rpm for 10min. The supernatant was precipitated with 45% ammonium sulphate. Dialysis was followed in a tubular cellulose membrane against 2L distilled water for 24hrs at 4°C . Antimicrobial activity of partially purified bacteriocins was carried out by following the agar spot test method by (14). The bacteriocin (BacPC) was purified to homogeneity by a four-

step protocol involving ammonium sulfate precipitation, centrifugal microconcentrators with a 10-kDa membrane cutoff, gel filtration Sephadex G-25 (15, 16, 17).

2.3.4. Protein Separation - SDS-PAGE- (17)

The proteins were separated by SDS-PAGE electrophoresis and size of polypeptide chains of given protein can be determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility marker proteins of known molecular weight. Two polyacrylamide gels were prepared and loaded with aliquots of each partially purified supernatant. After electrophoresis, gels were subjected to different treatments: one gel was fixed and stained with Coomassie blue solution to determine the peptide sizes using a Spectra Multicolor Low Range Protein Ladder.

The other gel was fixed in 20% (v/v) isopropanol and 10% (v/v) acetic acid solution for 30 min at room temperature, followed by rinsing for 2 h in MilliQ water and then left in MilliQ water overnight while shaking. The following day, the gel was rinsed with MilliQ water for 30 min, placed onto a thin layer of MRS agar and overlaid with 0.7% (w/v) MRS soft agar containing *S. aureus* as a sensitive indicator organism. The gel was then incubated for 24 h at 30°C followed by inspection for inhibition zones.

2.3.5. Determination of the minimal inhibitory concentration (MIC)

The minimal inhibitory concentrations (MIC) of the bacteriocin (BacPC) was determined in 96-wells plates (Germany) containing 100 μ L of the tested peptides. Phosphate buffer solutions (5 mM, pH 6.5) containing the nine combinations 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0 mg. L⁻¹ of (BacPC) was prepared.

For each combination, 100 μ L of the appropriate 2X culture media inoculated with the susceptible strains with an initial optical density value at 600 nm of 0.02 were added. Inhibitory activities of these bacteriocin(s) suspensions were determined by measuring O.D600 values after 18 h of incubation at the optimal temperature of each tested culture, assays were performed in triplicate (5).

2.4. Efficacy of protective lactic acid bacteria in growth control of *S. aureus* on the goat's milk

Commercial goat's milk was heat treated at 90°C for 15 min and was inoculated with overnight-grown culture of *Pc. pentosaceus* at 2 mL/100 mL, *S. aureus* at 10⁸ CFU/mL was also simultaneously added to the milk. Fermentation was carried out at 37°C for 12 h and the change in the number of viable cells of *S. aureus* was measured at 2 h intervals. To determine the number of the indicator strain, a 1 mL aliquot of the sample was withdrawn and immediately chilled on ice, diluted and plated onto Braid Parker agar. The plates were incubated at 37°C for 48 h and the colonies developed were counted (18).

3. Results and discussion

The search for new bacteriocins with a wider spectrum of activity which can be used in human health, agriculture, and the food industry is being studied by several research groups. Bacteriocin (BacPC) was an efficient antimicrobial agent against the foods pathogens microorganisms (8, 9). Bacteriocin (BacPC) (Table.01) was considered to be extremely heat stable as antibacterial activity was not altered by heat treatment after 15 min at 121°C. The pH stability was studied in the range of pH 2-12. It was observed that this bacteriocin was active at pH values from 2 to 12, and it was completely destroyed after 75 minutes exposure to UV light. pH adjustment to 6.5 was chosen to eliminate the possible effect of organic acids. Some studies of characterization of bacteriocins show that these molecules can be active under certain ranges of temperature and pH. Thermal processing is used extensively within the food manufacturing process and can have adverse effects on the bio-active capability of a bacteriocin, potentially rendering it less effective. The chemical and physical properties of a food, e.g. pH, and fat content, can also have a significant role in the suitability of a particular bacteriocin (5). The heat stability of bacteriocin discussed here indicates that it could be used as biopreservative in combination with thermal processing to preserve the food products (pasteurization, drying, and freezing). Our pH and temperature results were consistent with those reported by (19, 7, 20, 21). Bacteriocin (BacPC) was destroyed after exposure to UV light; these results confirmed the proteins status of the bacteriocin (12, 22, 23). The preservation capacity of the bacteriocin in terms of the periods and temperature of storage (Table. 02) was quite interesting, as it maintaining full stability for three months at 4°C and -20°C, indicating that the stability of bacteriocin to different conditions reflects that such compounds can withstand the conditions normally encountered in food processing so would remain effective during processing (19,24).

SDS-PAGE analysis of the purified bacteriocin (BacPC), revealed a single band with an estimated molecular mass 19 kDa, indicating that this bacteriocin has been purified to homogeneity (Fig. 1a). Antibacterial activity determination against *S. aureus* strain reveals a growth inhibitory zone at the same position than that visualized in the stained gel (Fig. 1b). The optimal bacteriocin recovery was achieved using ammonium sulphate precipitation; this is in conformity with the report of (25) that optimal bacteriocin recovery can be achieved by ammonium phosphate precipitation. Ultra filtration experiments in this study showed that bacteriocin were able to pass through the cut off membrane. A tendency to aggregate with other proteins has been reported in bacteriocins produced by other lactic acid bacteria (26) and might have contributed to why the bacteriocins could not pass through the membranes with low molecular weight cut off. Some bacteriocins appear in their native state as aggregates with high molecular mass (ca. 30-300 kDa). These aggregates may mask partially or completely the antimicrobial activity of the bacteriocins during their purification and also induce errors in the determination of their molecular weight. This is especially true with highly non-polar low-molecular-weight bacteriocins, which easily interact with extracellular material of lysated cells (e.g., cell wall debris and micelles of lipoteichoic acids) and other non-polar compounds from the culture medium (27). Once the bacteriocins are recovered from the cell-free supernatants, they can be concentrated by techniques permitting separation of the fractions according to their size and/or physicochemical properties (27). Molecular weight of the bacteriocin produced by *Lactobacillus* sp. MSU3IR was 3-3.5kDa after purification with ammonium sulfate precipitation, SDS-PAGE, anionic chromatography and RP-HPLC (28). This result was close with that obtained from the bacteriocins like BacTN635 produced by *L. plantarum* sp. TN635 with molecular mass of 4 kDa (17), and megacin A-19213; being composed of two subunits, one of which is about 7.5 kDa in mass, and to that reported for enterocin CRL35 (29). Other bacteriocins produced by *Pc. acidilactici* and *B. cereus* 8/10 where molecular weights were estimated to range from 3.5-6.5 and 4-6 kDa, respectively (11). The molecular weight of the purified bacteriocin BA28 was calculated to be about 6.4 kDa (30). According to (31), mesentericin Y105 is a 37-residue bacteriocin produced by *Leuconostoc mesenteroides* Y105 that displays antagonistic activity against Gram-positive bacteria such as *Enterococcus faecalis* and *Listeria monocytogenes*. It is closely related to leucocin A. Bacteriocins from *Pediococcus* species, designated as pediocins, were shown to be protein in nature and inhibitory to several other bacteria. Pediocin F, one of the bacteriocins produced by *P. acidilactici* isolated from fermented sausage, is a small peptide, with a molecular weight of approx. 4.5 kDa that has shown to be effective against many bacteria associated with food spoilage and food related health hazards (7). The partial purification of bacteriocin produced by *Lactobacillus* sp. MSU3IR had manifested the presence of two peptide bands with the molecular weight of 39.26 and 6.38 kDa, respectively (32). Bacteriocin BA28 produced by *Pc. acidilactici* BA28 was analyzed based on the Rf value of the markers and the Rf value of the peptide compounds in SDS – PAGE. After electrophoresis, it was recorded that the band 1 (AMP1) 16 kDa and the band 2 (AMP2) was having 32 kDa (30).

The minimal inhibitory concentration (MIC) of bacteriocin (BacPC) for the indicator organisms *S. aureus* was 6 mg.ml⁻¹ and MBC was 35 mg.ml⁻¹, this results were likely to be of considerable importance for the LAB producing these substances (5, 33).

Bacteriocin produced by *Pc. pentosaceus* sp. resulted in rapid inactivation of *S. aureus* from an initial population of 10⁸ CFU mL⁻¹ to a resistant population of 10³-10⁴ CFU mL⁻¹, when the strain was grown in association in the goat milk, the number of *S. aureus* gradually decreased and became undetectable at 12 h at 37°C. *Pc. pentosaceus* sp. appeared to be a suitable starter culture for preservation of the goat milk (Fig. 02). The results indicate that bacteriocin possessed several desirable characteristics of a biopreservative, similar results were obtained by *Lactobacillus* sp. and *Bacillus cereus* in juice (19), *Lactococcus lactis* LBII and *L. monocytogenes* in the pasteurized milk (34), *Lactococcus lactis*W8 and *L. monocytogenes* in fermented milk (Dahi) (18).

Table 01: Effect of heat, pH, and UV light on bacteriocin (BacPC) activity against the indicator strain *S. aureus*. Results are expressed as % of means values of growth reduction (n= 3) ± standard deviations

Temperature °C	30	60	90	120
Bacteriocin (BacPC)	40± 0.2	56± 0.2	73± 0.3	80± 0.2
pH	3	6	9	12
Bacteriocin (BacPC)	50± 0.5	50± 0.1	64± 0.2	52± 0.3
Time of UV light exposure (min)	15	30	60	75
Bacteriocin (BacPC)	4.60 ± 0.2	4.96 ± 0.2	4.64 ± 0.3	4.24 ± 0.2

Table 02: Effect of time and temperature of storage on bacteriocin (BacPC) activity against the indicator strain *S. aureus*. Results are expressed as % of means values of growth reduction (n= 3) ± standard deviations

Time of storage (days)	Bacteriocin (BacPC)	
	-20°C	4°C
15	50 ± 0.2	80 ± 0.1
30	50 ± 0.2	80 ± 0.2
45	55 ± 0.3	78 ± 0.2
60	48 ± 0.4	70 ± 0.1
90	46 ± 0.4	56 ± 0.1

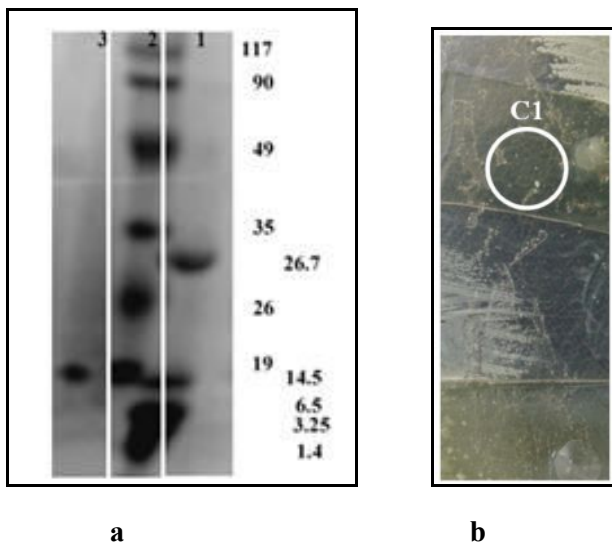


Figure 01. Electrophoretic and zymogram analyses of the purified bacteriocin (BacPC). Coomassie brilliant blue R-250 stained SDS-PAGE gel. Lane 1 and 2 molecular mass markers; lane 3 purified BacPC. Portion of the renatured SDS-PAGE, overlaid with MRS soft agar containing *S. aureus* (b).

Table 03: The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the bacteriocin (BacPC) against *S. aureus*.

	BacPC
MIC ^a (mg.l ⁻¹)	06 ± 0.5
MCB (mg.l ⁻¹)	35 ± 5

^aMIC. Each result represents the mean of three independent experiments.

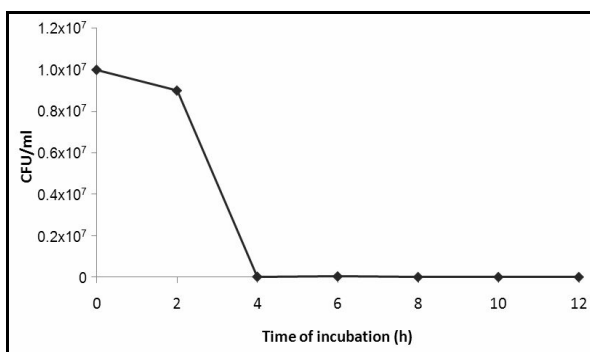


Figure 02: Reduction in population of *S. aureus* in goat milk with the addition of *Pc. pentosaceus* sp. as starter at 37°C.

4. Conclusion

The study revealed that bacteriocin (BacPC) from *Pc. pentosaceus* sp. isolated from algerian goat milk possesses a spectrum of inhibitory activity against the food-borne pathogen *S.aureus*. Therefore, it has a potential for application as a biopreservative in the dairy milk products. Since lactic acid fermentation is employed mostly for development of products, especially for flavor and taste of the fermented products, for enhancing the microbial quality and safety of processed foods.

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6. References

1. Giri S. and Singh J., New Face in the Row of Human Therapeutics: Bacteriocins School of Biotechnology. J Microbiol Res., 2013, 3, 2, 71-78.
2. Rattanachaikunsopon P. and Phumkhachorn P., Lactic acid bacteria: Their antimicrobial compounds and their uses in food production. Ann Biol Res., 2010, 1, 4, 218-228.
3. Lika H.M.Å., Shatha A.S.Å. and Hamzia A.A.Å., Effects of crude and purified bacteriocin of *Pediococcus pentosaceus* on the growth and zearalenone production by *Fusarium graminearum*. Int J Cur Eng Tech., 2014, 4, 12347-5161.
4. De Vuyst L. and Leroy F., Bacteriocins from lactic acid bacteria: production, purification, and food applications. J Mol Microbiol Biotechnol., 2007, 13, 4, 194-9.
5. Jasniewski J., Cailliez-Grimal C., Gelhaye E. and Revol-Junelles A.M., Optimization of the production and purification processes of carnobacteriocins Cbn BM1 and Cbn B2 from *Carnobacterium maltaromaticum* CP5 by heterologous expression in *Escherichia coli*. IOSR J Pharm., 2008, 3, 44-50.
6. Moshood A.Y., Lactic Acid Bacteria: Bacteriocin Producer: A Mini Review. International Islamic University Malaysia, Bandar Indera Mahkota, Jalan Istana, 25200 Kuantan, Malaysia., 2013.
7. Parada J.L., Caron C.R., Bianchi P.A., Ricardo Soccol M. and Ricardo Soccol C., Bacteriocins from Lactic Acid Bacteria: Purification, Properties and use as Biopreservatives. Brazilian Arch Biol Technol., 2007, 3, 50, 521-542.
8. Djadouni F. and Kihal M., Characterization and determination of the factors affecting anti-listerial bacteriocins from *Lactobacillus plantarum* and *Pediococcus pentosaceus* isolated from dairy milk products. African J Food Sc., 2013, 7, 2, 35-44.
9. Djadouni F. and Mebrouk K., Antimicrobial Activity of Lactic Acid Bacteria and the Spectrum of their Biopeptides against Spoiling Germs in Foods. Brazilian Arch Biol Technol., 2012, 55, 3, 435-443.
10. Budde B.B., Hornbaek T., Jacobsen T., Barkholt V. and Koch A.G., *Leuconostoc carnosum* 4010 has the potential for use as a protective culture for vacuum-packed meats: culture isolation, bacteriocin identification, and meat application experiments. Int J Food Microbiol., 2003, 83, 171-184.
11. Albano H., Todorov S.D., Van reen C.A., Hogg T., Dicks L.M. and Teixeira P., Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated from Alheira, a fermented sausage traditionally produced in Portugal. J Food Microbiol., 2007, 116, 239-247.
12. Ogunbanwo S.T., Sanni A.I. and Onilude A.A., Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African J Biotechnol., 2003a, 08, 2, 219-227.
13. Wanda J. and Bonita A., Partial purification and characterization of a bacteriocin produced by *Propionibacterium thoenii*. App J Env Microbiol., 1991, 57, 701-706.
14. Schillinger U. and Lüke F., Antimicrobial activity of *Lactobacillus sake* from meat. App Env Microbiol., 1989, 55, 1901-1906.
15. Bradford M.M.A., A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 1976, 72, 248-254.
16. Piva A. and Headon R., Pediocin A. Bacteriocin produced by *Pediococcus pentosaceus* FBB61. J Microbiol., 1994, 140, 697-702.
17. Smaoui S., Elleuch L., Bejar W., Karray-Rebai I., Ayadi I., Jaouadi B., Mathieu F., Chouayekh H., Bejar S. and Mellouli L., Inhibition of Fungi and Gram-Negative Bacteria by Bacteriocin BacTN635 Produced by *Lactobacillus plantarum* sp. TN635. App Bioch Biotechnol., 2009, DOI 10.1007/s12010-009-8821-7.

18. Suranjita Mitra a., Pran Krishna Chakrabartty b. and Swadesh Ranjan Biswas a., Potential production and preservation of dahi by *Lactococcus lactis* W8, a nisin-producing strain. Indian J Food Sc Technol., 2010, 43, 337-342.
19. Joshi V.K., Sharma S. and Rana N., Production, purification, stability and efficacy of bacteriocin from isolates of natural Lactic acid fermentation of vegetables. Indian J Food Technol Biotechnol., 2006, 3, 44, 435-439.
20. Kouakou P. and Thonart P., Action des cultures protectrices: Cas des germes lactiques sur la flore alimentaire indésirable. Env Soc Biotechnol Agr., 2011, 2, 15, 339-348.
21. Labioui H., Elmoualdi L., Ouhssine M. and Elyachioui M., Evaluation de l'activité antibacterienne des bactéries lactiques bactériocinogènes et leur application dans la biotransformation des déchets des abattoirs. Labo Biotechnol Microbien, Kenitra., 2010, 01.
22. Khalil R., Djadouni F. and Omar Sanaa., The influence of cultural and physical conditions on the antimicrobial activity of bacteriocin produced by a newly isolated *Bacillus megaterium* 22 strain. African J Food Sc., 2009a, 3, 1, 011-022.
23. Khalil R., El Bahloul Y.A., Djadouni F. and Hamed Omar S., Isolation and characterization of a bacteriocin produced by a newly isolated *Bacillus megaterium* 19 Strain. Pakistan J Nutr., 2009B, 3, 8, 242-250.
24. Gálvez A., Hikmate A., Lucas López R. and Ben Omar N., Strategies for food biopreservation. Int J Food Microbiol., 2007, 120, 51-70.
25. Ivanova I., Kabadjova P., Pantev A., Danova S. and Dousset X., Detection, purification and partial characterization of a novel bacteriocin substance produced by *Lactococcus lactis* subsp. *lactis* B14 isolated from Boza Bulgarian traditional cereal beverage. Biocatalysis France., 2000, 47-53.
26. Toba T., Yoshioka E. and Itoh T., Lacticin, a bacteriocin produced by *Lactobacillus delbrueckii* subsp. *Lactis*. Lett App Microbiol., 1991a, 12, 2, 43-45.
27. Cintas L.M., Herranz C., Hernández P.E., Casaus M.P. and Nes L.F., 2001. Review: Bacteriocins of lactic acid bacteria. Food Sci Tech Int., 2001, 7, 281-305.
28. Susana R., Márcia C.C., Airidas D., Maria L.E.D. and Célia C.G.S., Isolation and characterization of bacteriocins produced by lactic acid bacteria. Portugal., 2013.
29. Wachsmann M., Kulka M., Smith C.C. and Aurelian L., A growth and latency compromised herpes simplex virus type 2 mutant (ICPIODeltaPK) has prophylactic and therapeutic protective activity in guinea pigs. Vaccine., 2003, 28, 1879-1890.
30. Kaur B. and Garg N., Characteristics of bacteriocin BA28 produced by *Pediococcus acidilactici* BA28. Min J Pharm Medical Sc., 2013, 17-20.
31. Morisset D. and Frere J., Heterologous expression of bacteriocins using the mesentericin Y105 dedicated transport system by *Leuconostoc mesenteroides*. Bioch., 2002, 84, 5-6, 569-576.
32. Palanisamy I., Thirumalai M., Ramasamy R., Santhiyagu P., Chandrasekaran K., Grasian I. and Arunachalam P.I. et al., Optimization of bacteriocin production by *Lactobacillus* sp. MSU3IR against shrimp bacterial pathogens. Aqu Biosy., 2013, 9, 12.
33. Bharal A. and Kumar V.S., Evaluation of antimicrobial activity of bacteriocin (*L. acidophilus*) against human pathogenic and food born microorganisms. Int J Innov Res Sc Eng Tech., 2013, 2, Issue 9.
34. Benkerroum N., Behavior of *Listeria monocytogenes* and *Staphylococcus aureus* in yogurt fermented with a bacteriocin-producing thermophilic starter. J Food Protect., 2002, 65, 799-805.
