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Purification and Characterization of Bacteriocin producing Bioactive Compounds from soil origin *Pseudomonas sp*.

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Several bacterial microorganisms were isolated from brown garden soil and were identified at the genus level through Gram-stain and biochemical characterization. The identified cultures were later checked for its bioactive compound activity against the food contaminants such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Clostridium perfringens* isolated from cooked chicken waste. Of these tested cultures against the contaminants, only *Pseudomonas sp*. was able to produce bioactive compounds rendered as bacteriocin. *Pseudomonas sp*. was analyzed using 16S rRNA sequencing technique and was identified as *Pseudomonas monteillii*. The identified bacterial strain was mass-cultured and partially purified using ammonium sulfate, centrifuged and the collected pellet was dissolved using equal amount of PBS (Phosphate buffer saline) solution. The collected crude protein sample was purified using column chromatography carried out at different fractions. The purified fraction was then characterized using SDS-PAGE analysis. The protein band was cut and analyzed using MALDI-TOF/MS analysis technique to confirm the presence of accurate bioactive peptide mass fragments in the purified protein sample.

Key words: *Pseudomonas monteillii*, Bacteriocin, 16S rRNA, Ammonium Sulfate, SDS-PAGE, MALDI-TOF/MS.

1. Introduction

Bioactive compounds are peptides produced by various bacteria with antimicrobial activity against different microorganisms (1). These compounds with use of hydrophobic/amphiphilic properties penetrate into the cell and consequently the bacterial compositions release from the cell (2). However, production of bioactive compounds such as bacteriocins is similar to antibiotics but several advantages could be considered when compared to the antibiotics. For example, toxicity is relatively nil. In addition, proteinaceous property of these compounds resulted in their degradation by protease enzymes; hence could not accumulate in the human body (3). In general, bacteriocins are divided into four groups based on their structures. Class I bacteriocins are small peptides (<5 kDa) with unusual amino acids. Class II are thermo stable peptides with size of <10 kDa. Class III are heat-sensitive with high molecular weight (<30 kDa) and Class IV are relatively complex with glycoprotein, lipoprotein and non-protein moiety (4). It is confirmed that non-protein moieties of bacteriocins are required for stability and antimicrobial activity (5).

Nowadays, applications of bacteriocins are considered as a way to reduce the frequency of occurrence of antibiotic-resistant bacteria. For example, alternative drugs as well as safe food preservatives could eliminate antibiotic-resistant strains of bacteria and increase the shelf life of food (6). The food preservation through natural methods represents one of the main concerns at the global level to solve economic losses due to microbial decomposition of raw materials and foodstuffs. With concomitant expansion of the research, commercial, food industry and medical sectors, the field of biopreservation using probiotic bacteria is developing rapidly with accumulation of many data about their benefits. Although many bacteria produce antimicrobial substances, the benefits of those produced by Lactic Acid Bacteria (LAB) is of particular interest because of their Generally Recognized As Safe (GRAS) status which acts as natural biopreservative and natural flavor enhancers. Hence, the majority of antimicrobial peptide-producing LAB is ideally suited to food applications, therefore, the production of bacteriocins by LAB is not only advantageous to the bacteria themselves but

could also be exploited as a tool to food industries for controlling undesirable bacteria in a natural manner, and be allowable to the consumer.

Nowadays many investigations are focused on discovering novel bacteriocins for controlling the undesirable bacteria in food products (2), (7). There is a need to attract consumer attention to natural substances rather than conventional synthesis of chemical one as protector against pathogens. As probiotics has been accepted in the market for their beneficial properties, and in the same way, the bacteriocin producing probiotic strains should become attractive especially to natural food preservation. Continued research on bacteriocins will undoubtedly lead to our increased understanding, and with the emergence of new bacteriocins, new potential bio-preservatives.

2. Materials and Methods

2.1 Collection of Soil Sample

A heap of brown loam garden soil was collected in a sterile glass beaker from the campus within Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

2.2 Isolation of Bacteria

About 8 bacterial strains were isolated from the collected soil sample. These strains were isolated using serial dilution technique starting from 10-2 to 10-8 dilution. Each of the isolated culture were grown in specific media and was differentiated based on Gram staining and Biochemical characterization.

2.3 Assay Activity for Bioactive Compounds Production

The isolated bacterial strains were analyzed for the production of bioactive compounds by crushing the cooked chicken waste using sterile water from where few bacterial food pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Salmonella abony* and *Clostridium perfringens* using serial dilution technique and were tested against the isolated 8 soil bacterial strains using spread plate technique which was incubated for a period of about 24 to 48 hours and observed for the zone formation. The food borne pathogens were spread onto the Muller Hinton Agar plates using sterile cotton swab. Well was created within the petri-dishes and all the 8 bacterial isolates in broths were added to each of the wells at different concentrations (20µl, 40µl, 60µl and 80µl) and kept for 24 to 36 hours of incubation. After the stipulated incubation time, the zone formation was formed around the wells of all the different concentrations and the inhibition zone values were recorded.

2.4 Selection of *Pseudomonas sp*.

Based on the visualization of zone formation, only *Pseudomonas sp*. was able to exhibit the bioactive compound production with a zone formation of diameter 3 cm among all the other soil bacterial isolates and was thus selected for further research. *Pseudomonas sp*. was sub-cultured and preserved in glycerol stocks at -4ºC for future works.

2.5 16S rRNA Sequencing

The bacterial genomic DNA was extracted from the chosen *Pseudomonas sp*. isolated using the procedure of Thermo Scientific Genomic DNA Purification Kit Catalog #K0512. 10µl of the reaction mixture was analyzed by submarine gel electrophoresis using 1.0 % agarose with ethidium bromide (0.5µg/µL) as per the standard protocols (8) at 80V/cm and the reaction product was visualized under gel documentation System (Alpha Innotech).

The 16S rRNA gene fragment was amplified using MJ Research PTC-225 Peltier Thermal Cycler and by employing two universal primers, p27F (5'-AGA GTT TGA TCM TGG CTC AG -3') and p1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). 1 µl of template DNA was added in 20 µl of PCR reaction solution and 35 amplification cycles were performed at 94ºC for 45 seconds, 55ºC for 60 seconds and 72ºC for 60 seconds. The PCR product was sequenced using the 518F/800R primers and the sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

The fluorescent-labelled fragments were purified using ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The sequence data was aligned and analyzed for identifying the bacteria using BLAST analysis and NCBI tools. The phylogenetic tree was also constructed using some bioinformatics software.

2.6 Bradford Protein Assay

This method was carried out by Fanglian He (9). Five to eight dilutions of a protein (usually BSA) standard with a range of 5 to 100µg protein was prepared. 30µl each of this unknown protein sample was added to an appropriately labelled test tube. Two blank tubes were set up. For the standard curve, 30µl of the distilled water was added while for the unknown protein samples, 30µl of protein preparation buffer was added. 1.5ml of Bradford reagent was added to each tube, mixed well and incubated at room temperature for at least 5 minutes (< 1 hour). The absorbance was measured at 595 nm.

2.7 Media Optimization

The liquid culture of *Pseudomonas monteillii* was experimented for media optimization using different parameters under optimal physical and chemical conditions such as Temperature, pH, Carbon sources and Nitrogen sources. The optical

density (OD) values were observed at an absorbance of 595nm using spectrophotometer for a period of every 2 hours until time duration of 24 hours and the results were tabulated down.

2.8 UV-Vis Spectrophotometer

The ultraviolet-visible spectroscopy (UV-Vis) utilizes light to determine the absorbance or transmission of a chemical species in either solid or aqueous state (Lizbeth Rostro, 2012). The crude sample of *Pseudomonas monteillii* culture was subjected to UV-Vis spectrophotometer and the spectral values were noted down at a wavelength ranging from 224nm to 1976nm.

2.9 Partial Purification of *Pseudomonas monteillii*

Freshly prepared 24 hours culture of *Pseudomonas monteillii* (5ml) was inoculated into the specific media (broth of 500ml) prepared using Pseudomonas isolation broth to produce the culture in a bulk amount also called as mass culturing. The culture was partially purified using ammonium sulphate at a concentration of 60% saturation.

2.10 Column Chromatography

The crude protein was experimented with column chromatography to remove the excess salt impurities present in the liquid sample using Sephadex-G-50 (10g) and was loaded and packed into 100 cm column of 150 x 10 mm bed volume. A total of 8 fractions were collected, analyzed for its antimicrobial activity and tabulated.

2.11 Antibacterial Assay

Each of the previously mentioned 8 fractions were checked for antibacterial activity using agar well diffusion assay by testing them against the overnight grown different bacterial cultures such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella abony*.

2.12 SDS-PAGE and MALDI-TOF-MS

The molecular weight of the purified protein obtained from the fraction of column chromatography was estimated by using Laemmli SDS-PAGE protocol (10). The gel was prepared and set at 100V for one and a half hours, washed for 1-2 minutes at room temperature, documented using E-gel imager (Lifetechnolgies, USA). The same sample was then exposed to Matrix Assisted Laser Desorption Ionization (MALDI) - Time of Flight (TOF) - Mass Spectrometric (MS) analysis to check and confirm the presence of accurate bioactive peptide masses and peptide fragment masses.

3. Results

3.1 Gram-Staining

Gram staining was performed to all the eight soil bacterial isolates and viewed under 100X (Table 1).

3.2 Selection of *Pseudomonas sp*.

Of the isolated 8 bacterial cultures only *Pseudomonas sp*. was able to exhibit the zone formation (bioactive compound production) against the food borne pathogens and it was chosen for further research activities (Figure 1).

3.3 Identification of *Pseudomonas sp*.

A series of biochemical tests were performed for the identification and confirmation of *Pseudomonas sp*. (Table 2) and a phylogenetic tree was created to identify *Pseudomonas sp*. based on 16s rRNA gene sequence analysis (Figure 2). The bacterium was identified as *Pseudomonas monteilli*. For further analysis, the bacterial culture of *Pseudomonas monteillii* was subjected to Agarose Gel Electrophoresis (AGE) to isolate and quantify the purity of DNA.

3.4 Media Optimization

Media optimization of the crude bacterial culture of *Pseudomonas monteillii* was analyzed to view its growth and stability under different optimum physiochemical parameters (Tables 3-4).

3.5 UV-Vis Spectrophotometer

The bacterial culture *Pseudomonas monteillii* was exposed to UV-Vis spectrophotometer and the readings were graphically represented (Graph 1).

3.6 Column Chromatography

After the partial purification, the centrifuged intracellular cells were dissolved in Phosphate Buffer Saline (PBS) solution, partially dialyzed and then purified using Sephadex G-50 solvent. The results were tabulated in the form of purified fractions (Table 5).

3.7 Antibacterial Assay

Among all the 8 collected fractions of the purified column chromatography samples, only the 5th fraction exhibited the zone of inhibition (Figures 3 (A), (B), (C)).

3.8 SDS-PAGE and MALDI-TOF-MS

The fraction with the higher antimicrobial activity was examined for protein isolation. Here only a single band was detected which confirmed the purity of the bacterial peptide. The molecular weight of the purified protein was revealed as 18.82 kDa in comparison to the marker used (Figure 4). The alignment of the particular peptide sequence was made using UNIPROT BLAST to identify the peptide sequence and the results showed the

complete homology of bacteriocin protein sequence that was expressed by various species of Pseudomonas bacterial culture (Graph 2). Based on MASCOT database search software the amino acid sequences of the known protein were predicted (Figure 5).

4. Discussion

Bacteriocins are bacterially produced peptides that are active against other bacteria and against which the producer has a specific immunity mechanism (11), (12). They are produced by all major lineages of bacteria and archaea and constitute a heterogeneous group of peptides with respect to size, structure, mode of action, antimicrobial potency, immunity mechanisms and target cell receptors (13). In general, Gram negative bacteria are usually considered to be resistant to many of bacteriocins from Lactobacillus strains (14). The bacteriocin production was found to be significantly influenced by pH, temperature, incubation time and other environmental factors (15), (16). The results obtained in this study revealed that the optimal production of bacteriocin from *Pseudomonas monteillii* occurs at pH 7, whereas many studies have revealed the bacteriocin production ranging between pH 4 and pH 5 (17) (18) and (19). The optimum temperature for the bacteriocin production from *E. faecium* was 35°C (16). The optimal production (12800AU/mL) of bacteriocin ST712BZ was recorded when incubated at 30°C but only 6400 AU/Ml was recorded when the cells were incubated at 37°C (20). In the present study, *Pseudomonas monteillii* was found to be stable at a temperature range of 45°C and pH 7 with the OD values of 1.28 and 1.98 respectively.

The bacteriocin producing *Lactobacillus plantarum* strain was isolated from the raw cow's milk samples that showed a broad range of antibacterial activity against various food borne pathogens (21). An increased amount of biological activity was also reported during purification of other bacteriocin in the pediocin family and may be due to the presence of some inhibitory compound at an earlier stage of the purification (22). Many investigators reported that different molecular mass were higher than the bacteriocin from ST16Pa (6.5kDa) (23). Here in this present study the molecular weight of the purified bacteriocin using SDS-PAGE was found to be 18.82 kDa.

The antibacterial spectrum of the isolate, partial purification, characterization and effect of some physical and chemical factors on the activity of bacteriocin from Leuconostoc NT-1 that inhibits several pathogenic and spoilage-causing bacteria were initiated by Maurya and Thakur (24). The inhibitory activity against some potent pathogens such as *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* is found to be an interesting feature of the bacteriocin. However the work carried out in this study revealed that *Pseudomonas monteillii* was found to exhibit the antimicrobial activity rendered as bacteriocin production against the food bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella abony*.

Several methods have been proposed for determining the binding affinity of antimicrobial peptides (AMPs) to bacterial cells. MALDI-TOF-MS was proposed as a reliable and efficient method for high throughput AMP screening that are selective and specific to a wide range of Gram-negative and Gram positive bacteria providing a simple reliable screening tool to determine the potential candidates for broad spectrum antimicrobial drugs (25). The alignment of the particular peptide sequence was carried out using UNIPROT BLAST and it has shown the complete homology of the bacteriocin protein sequence of various Pseudomonas sp. and based on the MASCOT search software and sequence homology analysis, the peptide was found to express the production of bacteriocin compound.

5. Tables, Figures and Equations

Table 1: Shows the list of Gram-Staining results of 8 isolated bacteria from brown loam garden soil

S.No.	Indicator Strains	Gram-Staining
1	*Escherichia coli*	-
2	*Salmonella abony*	-
3	*Staphylococcus aureus*	+
4	*Bacillus sp.*	+
5	*Pseudomonas sp.*	-
6	*Actinomycetes sp.*	+
7	*Klebsiella sp.*	-
8	*Vibrio harveyi*	-

Figure 1: Shows the bioactive compound production from *Pseudomonas sp*.

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Table 2: Shows the biochemical test analysis of *Pseudomonas sp.* culture

S. No	Biochemical Tests	Results
1.	Indole Test	–
2.	Methyl Red Test	–
3.	Vogues-Prausker Test	–
4.	Starch Hydrolysis Test	–
5.	Carbohydrate Fermentation Test	+
6.	Oxidase Test	+
7.	Catalase Test	+
8.	Spore-staining Test	+
9.	Urease Test	+
10.	Motility Test	+
11.	Gelatinase Test	+
12.	Citrate Utilization Test	+

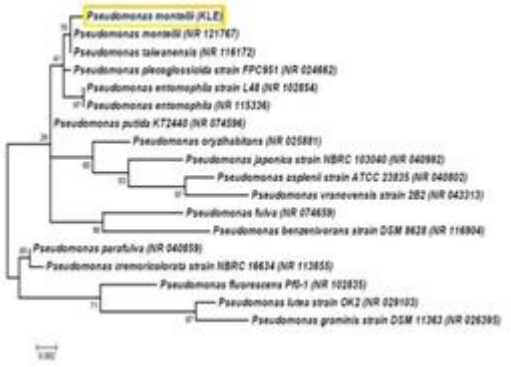


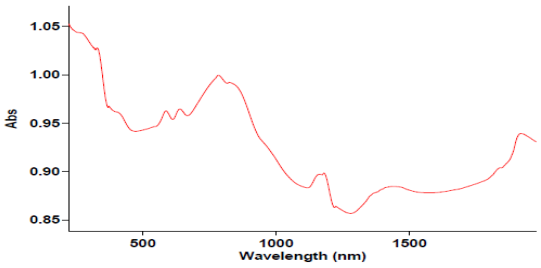
Figure 2: Phlogenetic tree of *Pseudomonas monteillii*

Table 3: Growth rate of *Pseudomonas monteillii* at different pH and Temperature ranges

pH	OD Value at 560nm	Temperature (T)	OD Value at 560nm
5	0.82±0.04	37	0.82±0.04
6	0.94±0.047	40	0.77±0.03
7	1.98±0.09	45	1.28±0.06
8	0.76±0.03	50	0.42±0.02

Table 4: Growth rate of *Pseudomonas monteillii* using different Carbon and Nitrogen sources

Carbon Source	OD Value at 560nm	Nitrogen Source	OD Value at 560nm
Glucose	1.10±0.05	Glycine	0.83±0.04
Sucrose	0.94±0.04	Urea	0.45±0.02
Maltose	0.31±0.01	Ammonium Sulphate	0.46±0.02
Galactose	0.50±0.02	Yeast Extract	0.39±0.01



(Wavelength vs Absorbance)

Graph 1: Shows the graphical representation readings of UV-Vis-Spectrophotometer

Table 5: Shows the list of 8 fractions collected using Column Chromatography

Fractions	OD at 595nm			Concentration (mg/ml)
	Duplicate 1	Duplicate 2	Avg.	
Free flow	0.1751	0.1812	0.17815	0.8369
1	0.2175	0.2072	0.21235	0.9969
2	0.1889	0.1903	0.1896	0.8901
3	0.1943	0.2069	0.2006	0.9418
4	0.2192	0.1947	0.20695	0.9716
5	0.2341	0.2092	0.22165	1.04061
6	0.1999	0.1999	0.1999	0.9385
7	0.2066	0.2044	0.2055	0.9648
8	0.2143	0.2029	0.2086	0.9793
Washout	0.1946	0.1976	0.1961	0.9206

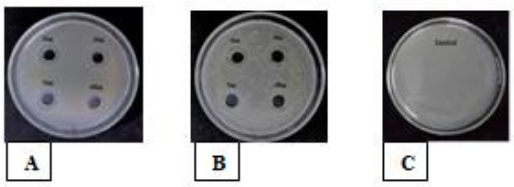


Figure 3 (A): Shows the antibacterial activity of 5th fraction of purified *Pseudomonas monteillii* against *E.coli*

Figure 3 (B): Shows the antibacterial activity of 5th fraction of purified *Pseudomonas monteillii* against *S.aureus*

Figure 3 (C): Shows the Control plate

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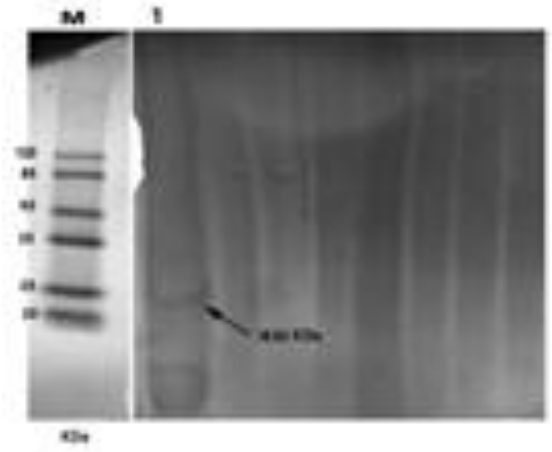
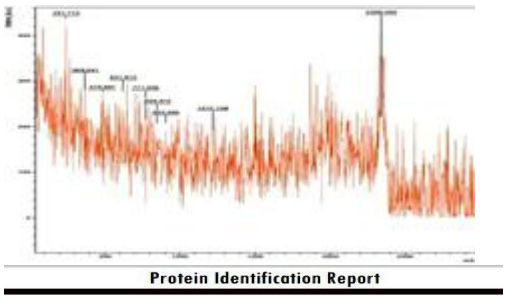


Figure 4: SDS-PAGE Analysis.
Lane M shows the marker values and
Lane 1 shows the purified protein band with a molecular weight 18.82 kDa



Graph 2: shows the mass to charge (m/z) ratio
Figure 5: Shows the fixed and variable modifications of the peptide masses using MASCOT software

6. Conclusion

Bacteriocin, a bioactive compound is an antimicrobial peptide that was isolated from the soil bacteria, *Pseudomonas monteillii*. The peptide was partially purified using ammonium sulphate with 60% saturation. The crude sample was then fractionized using column chromatography which resulted in collection of 8 purified fractions among which the 5th fraction exhibited the highest antimicrobial activity when compared with the other fractions. SDS-PAGE was used to purify and quantify the protein with a molecular weight of 18.82 kDa. Finally the presence of peptide fragments and its masses were expressed and confirmed using MASCOT search software through MALDI-TOF/MS analysis. Hence it can be concluded that the bioactive compound also rendered as bacteriocin was purified and characterized and possessed the efficiency to exhibit the antimicrobial activity against specific food-borne pathogens.

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Conflict of Interest

The authors confirm that the manuscript has no conflict of interest.

References

[1] Motta A S and Brandelli A: Evaluation of environmental conditions for production of bacteriocin-like substance by Bacillus sp. strain P34." World Journal of Microbiology and Biotechnology 2008, 24(5): 641-646.
[2] Oscáriz J C and Pisabarro A G: Classification and mode of action of membrane-active bacteriocins produced by gram-positive bacteria. International Microbiology 2001, 4(1): 13-19.
[3] Héchard Y and Sahl H G: Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria." Biochimie 2002, 84(5-6): 545-557.
[4] Ennahar S, Ishizaki A, Sashihara T and Sonomoto K: Class IIa bacteriocins: biosynthesis, structure and activity. FEMS Microbiology Reviews 2006, 24(1): 85-106.
[5] Garneau S, Martin N I and Vederas J C: Two-peptide bacteriocins produced by lactic acid bacteria. Biochime 2002, 84(5-6): 577-592.
[6] Settanni L and Corsetti A: Application of bacteriocins in vegetable food biopreservation. International Journal of Food Microbiology 2008, 121(2): 123-138.
[7] Adak G, Long S and O'Brien S J: Intestinal infection: Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut 2012, 51(6): 832-841.
[8] Sambrook J, Fritsch E F and Maniatis T: Molecular cloning: a laboratory manual, Cold spring harbor laboratory press 1989.
[9] He F: Bradford protein assay. Bio-protocol 2011, 1(6): e45.
[10] Laemmli U K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970, 227: 680–685.
[11] Cotter P D, Hill C and Ross R P (2005). Food Microbiology: Bacteriocins: Developing innate immunity for food. Nature Reviews Microbiology 2005, 3(10): 777-788.
[12] Klaenhammer T R: Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Reviews 1993, 12(1-3): 39-85.

[13] Gillor O, Etzion A and Riley M A: The dual role of bacteriocins as anti- and probiotics. Applied Microbiology and Biotechnology 2008, 81(4): 591-606.
[14] Todorov S D, Powell J E, Meincken M, Witthuhn R C and Dicks L M T: Factors affecting the adsorption of Lactobacillus plantarum bacteriocin bacST8KF to Enterococcus faecalis and Listeria innocua. International Journal of Dairy Technology 2007, 60(3): 221-227.
[15] Mortvedt-Abildgaa C, Nissen-Meyer J, Jelle B and Grenov B: Production and pH-Dependent Bactericidal Activity of Lactocin S, a Lantibiotic from Lactobacillus sake L45." Applied and Environmental Microbiology 1995, 61(1): 175-179.
[16] Annamalai N, Manivasagan P, Thangavel B and Vijayalakshmi S: Enterocin from Enterococcus faecium isolated from mangrove environment.. African Journal of Biotechnology 2009, 8(22): 6311-6316.
[17] Abo-Amer A: Molecular characterization of antimicrobial compound produced by Lactobacillus acidophilus AA11. Acta microbiologica et immunologica hungarica 2007, 54(2): 107-119.
[18] Usmiati S and Marwati T: Selection and optimization process of bacteriocin production from Lactobacillus sp. Indonesian Journal of Agriculture 2009, 2(2): 82-92.
[19] G.T. Tarelli G T, Carminati D and Giraffa G: Production of bacteriocins active against Listeria monocytogenes and Listeria innocua from dairy enterococci. Food Microbiology 1994, 11(3): 243-252.
[20] Todorov S D and Dicks L M: Bacteriocin production by Lactobacillus pentosus ST712BZ isolated from boza." Brazilian Journal of Microbiology 2007, 38(1): 166-172.
[21] Sankar N R, Priyanka V D, Reddy P S, Rajanikanth P, Kumar V K and Indira M: Purification and characterization of bacteriocin produced by Lactobacillus plantarum isolated from cow milk. International Journal of Microbiological Research 2012, 3(2): 133-137.
[22] Xiraphi N, Georgalaki M, Driessche G V, Devreese B, Beeumen J V, Tsakalidou E, Metaxopoulos J and Drosinos E H: Purification and characterization of curvaticin L442, a bacteriocin produced by Lactobacillus curvatus L442." Antonie Van Leeuwenhoek 2006, 89(1): 19-26.
[23] Todorov S D, Furtado D N, Saad S M, Tome E and Franco B D: Potential beneficial properties of bacteriocin producing lactic acid bacteria isolated from smoked salmon. Journal of Applied Microbiology 2011, 110(4): 971-986.
[24] Maurya A P and Thakur R: Inhibition spectrum, purification and characterization of bacteriocin from Leuconostoc NT-1.Current Science 2012, 103(12): 1405-1407.
[25] Mandal S M, Migliolo, L and Franco O L: The use of MALDI-TOF-MS and In-Silico studies for determination of antimicrobial peptides' affinity to bacterial cells. Journal of the American Society for Mass Spectrometry 2012, 23(11): 1939-1948.