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Original Article

Bacteriocin Production from Indigenous Strains of Lactic Acid Bacteria Isolated from Selected Fermented Food Sources

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ABSTRACT

Lactic acid bacteria (LAB) are a group of Gram-positive; non-spore forming, non-motile, non-respiring, bacteria produces variety of antimicrobial compounds such as lactic acid, acetic acid, ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins. Among them bacteriocines (a small molecular weight proteins) are in prime important due to their antimicrobial nature with food preservative abilities. Bacteriocins have gained a lot of attentions as bio-preservatives because of its GRAS status without causing any adverse effects on food. Nisin has been approved by US-FDA as a food preservative and is being used commercially worldwide by food industries. With these rationales, the aim of the present study is to produce bacteriocin (Nisin) from lactic acid bacteria isolated from selected fermented food sources, such as Curd, Mayonnaise and Jelly. Initially preserved lactic acid bacterial cultures were sub-cultured and their growth characters were studied on four different media namely MRS media, HJ media, KT media and DO media. Further seed culture of the selected bacterial species was prepared on the MRS broth (24hrs) and used as an inoculum for the production of bacteriocins. Later the 10% of the seed culture was inoculated to the 100 ml production media (CM media). After the 72 hrs of batch fermentation process, a crude extract of the fermentation broth was screened for the presence of Bacteriocins using agar well diffusion assay technique on *E.coli* and *Kleibshella sp* culture plates. All the three cultures of lactic acid bacteria showed antagonistic activity on the tested bacterial sp. Partial purification of the bacteriocin was done using ammonium sulphate precipitation and molecular weight characterisation of the obtained bacteriocins was done using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The results exhibited that, the bands representing the molecular weight less than 14 KDa and also the absorbance peak at 225 nm in UV spectra confirmed the presence of bacteriocin production. In conclusion in the present study attempt were made successfully in producing bacteriocine for indigenous cultures of LAB isolated from selected fermented foods samples.

Keywords: Lactic acid bacteria (LAB), Bacteriocins, Antimicrobial Activity, *E.coli, Kleibshella sp* and SDS-PAGE.

1. INTRODUCTION

Gram-positive, non-spore forming, non-motile, non-respiring, bacteria which are either rod or coccus shape and poses negative catalase activity were characterized

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as Lactic acid bacteria (LAB)[1]**.** LAB exhibits similarities in their morphological, metabolic and physiological characteristics and during the fermentation of carbohydrates produces lactic acid as the major end-product[2]**.** *Lactobacillus, Pediococcus, Leuconostoc, Lactococcus, Enterococcus, Melissococcus, Streptococcus, Lactosphaera, Carnobacterium, Oenococcus, Tetragenococcus, Vagococcus* and *Weissella* genera were some of the well known species of LAB[3-4]**.** LAB is widely distributed in the nature and mainly habitats in materials of plant origin, human and animal cavities (mouth, genital, and intestinal and respiratory tract), water, juices, fermented foods (dairy products, meat, fish, vegetables, fruits, silage, and beverages), as well as spoiled food, sewage, and decomposing plant materials[5]. LAB's have been used in fermented foods due to their beneficial influence on nutritional, organoleptic, shelf-life characteristics and also used in food preservation where LAB's can acidify the food resulting in inhibition of spoilage and pathogenic bacteria[6]. Some LAB display crucial antimicrobial properties with respect to food preservation, safety and also has the potential to combat gastrointestinal pathogenic bacteria such as *Escherichia coli* and *Salmonella sp.*[7]. The antimicrobial compounds produced by LAB include lactic acid, acetic acid, ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins [8]**.**

Bacteriocins are small, ribosomally synthesized, antimicrobial proteins or peptides that are produced by many different bacterial species including members of LAB [9]. Bacteriocins possess inhibitory activity towards closely related bacteria, whereas producer cells are immune to their own bacteriocins[10]**.** Bacteriocin is believed to be safe for human consumption since it becomes inactive when treated with digestive enzyme in the stomach[11]**.** Bacteriocins produced by LAB have attracted much more importance for their application in food preservation and gained a lot of attraction as bio-preservatives because of their GRAS (generally recognized as safe) status without causing any adverse effects on food [12]. Food preservation is achieved by using either a bacteriocin producing starter culture or by applying the bacteriocin itself as food additive in its relatively pure form. So far, only one bacteriocin, Nisin, has been approved by FDA as a food preservative and is being used commercially worldwide by food industries[13]. Pediocin PA-1, after Nisin, is the most studied bacteriocin of LAB[14]. Several scientific groups worldwide have recognized its potential as a bio-preservative, especially for use in certain specific food [15].

Bacterial antibiotic resistance considered to be a raising issue due to the extensive use of classical antibiotics in treatment of human and animal diseases. As a result, multiple resistant strains appeared and spread causing difficulties and restricted the use of antibiotics. In order to control their use in food and feed products, one possible alternative is the application of some bacterial peptides as antimicrobial substances instead of antibiotics. Among them bacteriocins produced by Lactic acid bacteria have attracted attention, as they are active in a Nano molar range and have no toxicity**.** Bacteriocins were first discovered by A. Gratia in 1925. The first bacteriocin was called Colicine because it killed *E.coli.* LAB strains found to inhibit the activity of several gram positive and gram negative bacteria such as *Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes* and *Staphylococcus*[11]**.** Lactic acid bacteria isolated from curd were found to poses antimicrobial activity against *E.coli* and *Staphylococcus aureus.*

Bacteriocins have been produced from Lactic acid bacteria obtained from various sources but there are yet many other fermented food sources. The aim of the present study is to produce bacteriocin (Nisin) from lactic acid bacteria isolated from selected fermented food sources such Curd, Mayonnaise and Jelly. Initially preserved lactic acid bacterial cultures were sub-cultured and their growth characters were studied on four different media; further seed culture of the selected bacterial species was prepared on the MRS broth (24hrs) and used as an inoculum for the batch production of bacteriocins. Later the 10% of the seed culture was inoculated to the 100 ml of production media (CM media). After the 72 hrs of batch fermentation process, a crude extract of the fermentation broth was screened for the presence of nisin using agar well diffusion assay technique on *E.coli* and *Kleibshella sp* culture plates. All the three cultures of lactic acid bacteria showed antagonistic activity on the tested bacterial sp. Partial purification of the bacteriocin was done using ammonium sulphate precipitation and molecular weight characterisation of the obtained bacteriocins was done using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The results exhibited the bands representing the molecular weight less than 14 KDa and also the absorbance peak at 225 nm in UV spectra confirmed the presence of bacteriocins production. In conclusion present study attempt were successful in producing bacteriocin for indigenous cultures of LAB isolated from selected fermented foods samples.

Objective

To produce bacteriocin from Lactic acid bacteria isolated from selected fermented food sources of southern India.

2. MATERIAL AND METHODS

Revival and Subculturing of preserved LAB cultures: Preserved Lactic acid Bacterial cultures isolated from selected fermented food sources - Curd, Mayonnaise and Jelly were revived initially to MRS liquid broth and further subcultured on four different solid media such as HJ (Hogg and Jago) media, KT (kiuru and Tybek) media, DO (Dougles et al) media and MRS (Mann Rogosa and Sharpe) media plates (Table-1), incubated at 37 ^{o}C for 18-24 Hrs. Fully grown cultures were taken for further morphological and microscopic screening studies [16].

Table 1: Composition of different media used for the subculture of LAB isolates

Sl. No	Type of the media	Composition	Concentration (w/v)	% Agar	pH
1.	MRS	Himedia	5.515%	1.2%	6.2
2.	HJ	Tryptone	3%	1.2%	6.5
		Yeast extract	1%		
		Beef extract	0.2%		
		Lactose	0.5%		
		KH2PO4	0.5%		
3.	DO	Tryptone	1%	1.2%	6.5
		Yeast extract	0.3%		
		Glucose	0.2%		
		Sodium	1%		
		Glycerophosphate	0.1%		
		Tris	0.033%		
		Calcium Anhydride	0.1%		
4.	KT	Nutrient broth	1.5%	1.2%	6.5
		Tomato juice	10%(v/v)		
		Autolysed yeast	20%(v/v)		
		Lactose	0.5%		

Growth studies on different media: Fully grown cultures of all the three LAB isolates cultured on four different media were studied morphologically by screening different growth characteristics such as size, shape, color, no of colonies and growth rate of the colony [17-18].

Microscopic studies: Subcultures of all the three LAB isolates were studied microscopically applying standard Gram's staining procedure. Study was carried out to determine the purity and the stability of the selected LAB isolates [19-20].

Production of Bacteriocins by batch fermentation (SmF): Single colonies of the selected LAB isolates were transferred aseptically into 100 ml of seed culture media (MRS broth) (Table. 1), and incubated at 37°C for 18-24 hours. Further the 10 % of the seed culture of

each LAB isolates was inoculated in 100 ml of production media (CM media) composed-Sucrose (2.86%), Tryptone (0.5%), Yeast extract (1%), Tween 80 (0.3%), Magnesium sulphate (0.02%), Sodium Chloride (0.81%) K2HPO4 (1.91%) Ascorbic acid (0.05%) and Agar (1.2%) taken in four different 250 ml conical flasks. All the inoculated production media was kept at 37°C, 6.5pH, 150 rpm for 72 Hrs[21].

Separation of Bacteriocin Crude Extract: After 72 hours of incubation, the bacteriocin produced in the fermentation broth was separated by centrifugation (10000 rpm) for 21 minutes at 4°C. The supernatant obtained (also called, cell free supernatant (CFS), is transferred to a 250 ml Erlenmeyer flask and pellet is washed off. The pH of the CFS obtained from the fermentation broth, was adjusted to 7.0 with 3M NaOH in order to hydrolyze any inhibitory activity that can be offered by H+ ions. Further primary purification of the bacteriocin was carried form the CFS using a cellulose acetate filter syringe with 0.22 µm pore size (Millipore, USA). The filtrate consists bacteriocin was added with phosphate buffer in order to avoid antagonism by hydrogen Peroxide. Bacteriocin producers also, produce various organic acids during the stationary phase. Hence phosphate buffer (1X) is added to regulate the antagonistic activity of organic acids [22].

Screening of Antibacterial activity of bacteriocin by agar diffusion method: Bacteriocins are known to possess antimicrobial activity which can be of either broad spectrum (affects unrelated indicator genus) or specific spectrum activity (affects indicator strain of closely related genus). *Escherichia Coli and Kleibshella sp.* were used as an indicator strains. The inhibitory activity of bacteriocine on the indicator strains was performed using agar diffusion assay. Wells (diameter of 4mm, and depth 5mm) were punctured in the carpet cultures of indicators strain and approximately 300 µl of purified bacteriocin sample was added to respective sample wells. A Chloramphenicol disc was maintained as positive control and 0.9% saline was used as a Negative control. All the Petri plates were incubated for 24-36 hours. After the incubation time BT Assay (Also known as bacteriocin titer assay), was established and a zone of clearance is observed around the wells [9-23].

Purification of Bacteriocin: Crude extract of Bacteriocins was purified further in order to obtain a purest state of bacteriocins. Ammonium sulphate precipitation was carried out in further purification of bacteriocin. It is known that bacteriocins are proteinaceous in nature and ammonium sulphate precipitation was a standard downstream procedure for molecules possessing proteinaceous nature. Crude extract was treated with solid ammonium sulphate 40, 50, and 60% saturation. The mixtures were stirred for 2 h at 4°C and later centrifuged at 14,000 rpm for 1 h at 4°C. The pellet was resuspended in 25 ml of 0.05 M potassium phosphate buffer having pH 7.0. Dialysis was carried out against the same buffer for 12 h in spectrapor dialysis tubing. Assay of the bacteriocin activity was carried out and titer was determined [22].

Qualitative determination of purified bacteriocins: 10 ml of purified Bacteriocin samples obtained from the batch cultures of all the three LAB isolates was qualitatively studied by measuring the absorbance spectra in between 200-240nm in UV Visible, spectrophotometer with respect to standard Bacteriocin (Nisin) obtained for Anand Agriculture University, Anand, Gujarat, India. The absorbance maxima of the purified samples were compared with the absorbance maxima of authenticated standard bacteriocin (Nisin) sample [24].

Molecular weight determination of purified bacteriocins: Bacteriocins are small molecular weight compounds ranging from 10-200 KDa. Hence estimating the molecular weight of purified bacteriocin

of the fermentation broth of different Lab isolates (Curd, Mayonnaise and Jelly) is necessary to establish the characterization of bacteriocins. Bacteriocins being proteinaceous in nature and was estimated by SDS-PAGE which is a DSPT technique used to determine the molecular weight of bacteriocins [25-26].

3. RESULTS AND DISCUSSION

Revival and Subculturing of preserved LAB cultures: The preserved Lactic acid Bacterial cultures isolated from food sources i.e Curd, Mayonnaise and Jelly were revived on MRS liquid broth and further subcultured on four different solid media such as HJ (Hogg and Jago) media, KT (kiuru and Tybek) media, DO (Dougles et al) media and MRS (Mann Rogosa and Sharpe) media plates. Fully grown colonies on MRS media of all the three subcultured isolates were shown in Fig.1.

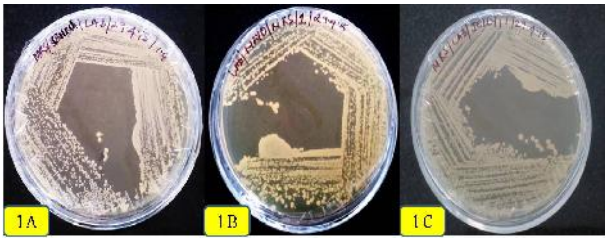

Fig 1: LAB colonies on MRS agar after 24 hrs of incubation.

1A) MRS Curd isolate, 1B) MRS Mayonnaise isolate, 1C) MRS Jelly isolate.

Growth studies on different media: The morphological studies considering the growth characters such as size, shape, color, no of colonies and growth rate of the colony, of subcultured LAB isolates of selected fermented food sources i.e - Curd, Mayonnaise and Jelly grown on four different solid media i.e HJ media, KT media, DO media and MRS were discussed in Table 2. It has been observed that growth was seen in all the media used and Jowar koozhu isolates was study in growth and exhibited high growth rates in all the four subcultured media. Studying the effect of various media on the growth of isolated LAB species is a primary aim of this study to screen the genetic and metabolic stabilities of the preserved indigenous LAB isolates.

Table 2: Growth studies of subcultured Lab isolates on different media

Sl no.	Source	Media	Size	Shape	Color	No of colonies	Growth rate
	Curd	HJ agar	Tiny	Round	White	2-3colonies	Low dense growth
	Mayonnaise	HJ agar	Big	Round	White	9 colonies	Fast dense growth
	Jelly	HJ agar	Tiny	Round	White	2-3colonies	Low dense growth
	Curd	DO agar	Tiny	Round	White	1-2 colonies	Steady growth
	Mayonnaise	DO agar	Moderate	Round	White	7-8 colonies	Dense growth
	Jelly	DO agar	Tiny	Round	White	1-2 colonies	Dense growth
	Curd	KT agar	Moderate	Round	White	5-6 colonies	Steady growth
	Mayonnaise	KT agar	Big	Round	White	7-8 colonies	Steady growth
	Jelly	KT agar	Moderate	Round	White	5-6 colonies	Steady growth
	Curd	MRS agar	Tiny	Round	White	6-7 colonies	Steady growth
	Mayonnaise	MRS agar	Big	Round	White	8-10 colonies	Steady growth
	Jelly	MRS agar	Tiny	Round	White	5-6 colonies	Steady growth

Microscopic studies: Grams stains of the subcultured LAB isolates of various selected fermented food sources i.e Curd, Mayonnaise and Jelly were shown in Fig.2.

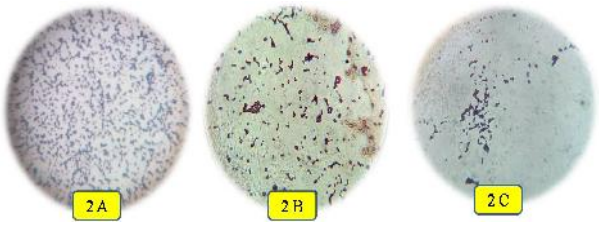

Fig 2: Gram's stain of LAB subcultures (100X).

1A).Curd isolates showing purple colored (Gram-positive) rods 1B). Mayonnaise isolates showing purple colored (Gram-positive) rods 1C). Jelly isolates showing purple colored (Gram-positive) rods.

Production of Bacteriocins by batch fermentation (SmF): The LAB isolates were grown under

submerged fermentation conditions to screen their potential for bacteriocin production. At the end of 72 Hrs of fermentation the acidified broth was takes for the purification of bacteriocines. The developed inoculum of the selected LAB isolates was shown in the Fig.3.

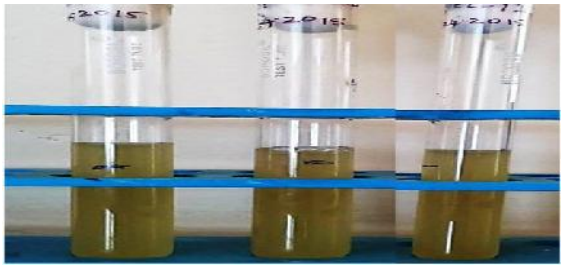

Fig 3: The developed inoculum (24 hrs) of the selected LAB isolates for different unexplored food sources i.e Curd, Mayonnaise and Jelly.

Separation of Bacteriocin Crude Extract: The extracted bacteriocins form the fermented broth of different Lab isolates were shown in Fig.4.

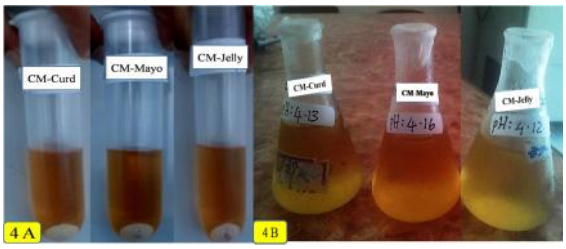

Fig 4: Separation of Bacteriocin Crude Extract.

A.) After centrifugation supernatant contains Bacteriocin 4B). Cell-free supernatant containing bacteriocin.

Screening of Antibacterial activity of bacteriocin by agar diffusion method: The crude bacteriocine extracts showing zone of inhibition on *E.coli* lawn cultures Fig.5. All the Crude extract tested were shown zone of inhibition on lawn cultures of both the tested indicator organisms. The amount of Bacteriocin affecting the indicator strain is given by AU (Arbitrary Unit)/ ml. One unit of AU is defined as the reciprocal of the diameter given by the highest serial dilution.

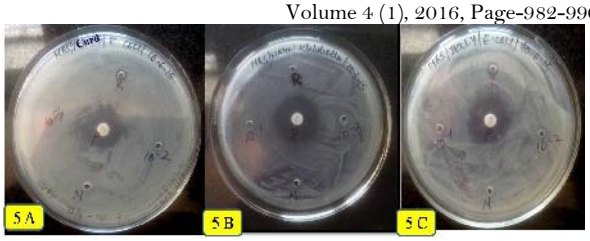

Fig 5: Screening of antibacterial activity of bacteriocin by agar diffusion method.

5A). Curd LAB SmF extract showing zone of inhibition on E. coli plates, 5B). Mayonnaise LAB SmF extract showing zone of inhibition on E. coli plates. 5C). Jelly LAB SmF extract showing zone of inhibition on E. coli plates.

Purification of Bacteriocin: Crude extract of Bacteriocins was purified further through Ammonium sulphate precipitation method and the purified bacteriocins of SmF cultures of LAB isolated from selected fermented food source such as Curd, Mayonnaise and Jelly, were shown in the Fig.6.

Fig 6: Purified bacteriocins of SmF cultures of LAB isolated from selected fermented food source such as Curd, Mayonnaise and Jelly.

Qualitative determination of purified bacteriocins: The qualitative confirmation of purified bacteriocin was done spectrophotometrically (three replicates). The samples and the standard exhibited a peak at 225 nm in the UV spectrophotometer scanning spectra (200-240 nm) shown in Fig 7. From the results, it was deduced that the all three LAB isolates i.e Curd, Mayonnaise and Jelly tested were found to be positive for bacteriocin production.

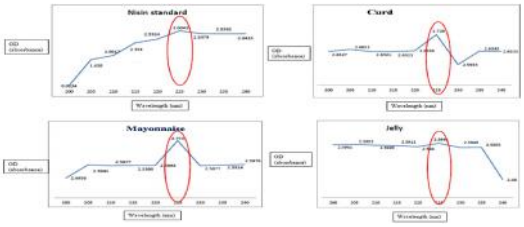

Fig 7: UV spectrophotometer scanning spectra (200-240 nm) of purified lovastatin.

Molecular weight determination of purified bacteriocins: The results of the SDS PAGE separation of purified bacteriocin was shown in Fig.8. The partial purified bacteriocin appeared as a diffused band in SDS-PAGE with molecular weight approximately less that 14 kDa (Fig. 8A & 8B). Similarly Ravi *et al*.,[26] reported the molecular weight of the bacteriocin from *L. plantarum* as 9.5 kDa. The bacteriocins of lactic acid bacteria belonging to class-I and II have molecular weight less than 10 kDa.

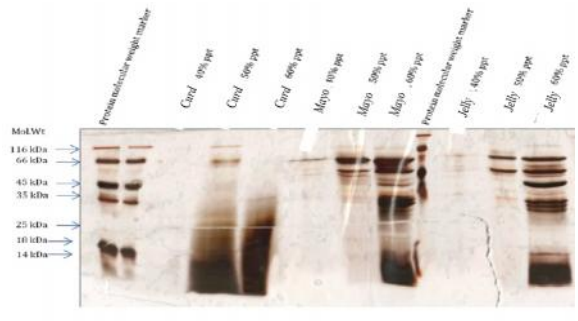

Fig 8: SDS PAGE gel showing the Molecular weight bands of different purified bacteriocins.

4. CONCLUSION

In the present study, initially LAB isolated from unexplored food sources such as Curd, Mayonnaise and Jelly were revived from the preserved cultures and screened for their growth and metabolic stability by morphological and microscopic examination. Further SmF process was carried out for the production of Bacteriocin. Purified Bacteriocins from the fermented broth was screened for their antibacterial activity and qualitatively confirmed by UV spectra (200-240nm) with the authenticated standard bacteriocin (Nisin). SDS-PAGE molecular weight studies (less than 14 kDa) also confirmed the presence of bacteriocins. In conclusion in the present study attempt were successful in producing bacteriocine for indigenous cultures of LAB isolated from different unexplored food samples.

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6. REFERENCES

1. Khalid Khalisanni. An overview of lactic acid bacteria. International Journal of Biosciences 2011; 1(3): 1-13.
2. Mayo B, D van Sinderen, and M Ventura (2008): Genome Analysis of Food Grade Lactic Acid-Producing Bacteria: From Basics to Applications. Current Genomics 2008; 9(3): 169–183.
3. Ali, Asmahan Azhari (2011). Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk in Khartoum State, Sudan. International Journal of Dairy Science 2011; 6: 66-71.
4. Khandelwal, P., Gaspar, F. B., Crespo, M. T. B., and Upendra, R. S. Lactic acid bacteria General Characteristics, Food preservation and Health Benefits'. In Lactic Acid Bacteria. Fermented Foods, Part I: Biochemistry and Biotechnology, CRC-press,2015; pp. 127-147.
5. König H and Fröhlich J. (2009). Lactic Acid Bacteria. In: König H, Unden G, Fröhlich J, editors. Biology of Microorganisms on Grapes, in Must and in Wine. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009; 3–29–29.
6. Nishant T, Sathish Kumar D, Arun Kumar R, Hima Bindu K, Raviteja Y. Bacteriocin Producing Probiotic Lactic acid Bacteria. Journal of Microbial & Biochemical Technology 2011; 3: 121-124.

7. Malik Deepak Kumar, Divya Bhatia, Anil Nimbriya and Sandeep Kumar. Lactic Acid Bacteria And Bacteriocin: A Review. Journal of Pharmacy Research 2012; 5(5): 2510-2513.
8. Jagoda Suskovi, Blazenka Kos, Jasna Beganovic, Andreja Lebos Pavunc, Ksenija Habjanic and Srecko Matosic. Antimicrobial Activity of Lactic Acid Bacteria, Food Technology, Biotechnology, 2010; 48 (3): 296–307.
9. Yang E, Fan L, Jiang Y, Doucette C, Fillmore S Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express 2012; 2(48):1-12.
10. De Vuyst L. Leroy F (2007): Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. Journal of Molecular Microbiology and Biotechnology 2007; 13:194–199.
11. Sharma Nivedita, Riti Kapoor, Neha Gautam and Ranjana Kumari. Purification and Characterization of Bacteriocin Produced by Bacillus subtilis R75 Isolated from Fermented Chunks of Mung Bean (Phaseolus radiatus). Food Technology, Biotechnology 2010; 49 (2): 169–176.
12. http://www.fao.org/docrep/meeting/028/mg309e.pdf.
13. Jeevaratnam K, Jamuna M, Bawa AS. Biological preservation of foods - bacteriocins of lactic acid bacteria. Indian Journal of Biotechnology 2005; 4:446–454.
14. Nikki Horn, María I. Martínez, José M. Martínez, Pablo E. Hernández, Michael J. Gasson, Juan M. Rodríguez, and Helen M. Dodd. Enhanced Production of Pediocin PA-1 and Coproduction of Nisin and Pediocin PA-1 by Lactococcus lactis. Applied and Environmental Microbiology 1999; 65(10): 4443–4450.
15. Yang Shih-Chun, Chih-Hung Lin, Calvin T. Sung, and Jia-You Fang. Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Frontiers in Microbiology 2014; 5: 241.
16. Sozzi, T., R. Maret, and J. M. Poulin. Study of plating efficiency of bacteriophages of thermophilic lactic acid bacteria on different media. Applied and environmental microbiology 1976; 32(1): 131-137.
17. Mohsen Zareian, Afshin Ebrahimpour, Fatimah Abu Bakar, Abdul Karim Sabo Mohamed, Bita Forghani, Mohd Safuan B. Ab-Kadir, and Nazamid Saari. A Glutamic Acid-Producing Lactic Acid Bacteriua Isolated from Malaysian Fermented Foods. International Journal of Molecular science 2012; 13(5): 5482–5497.
18. Upendra R. S, Pratima Khandelwal, Z. R. Amiri, Aparna. S, Archana. C and Ashwathi. M. Isolation and Characterization of lovastatin producing food grade fungi from oriental foods. World Journal of Pharmaceutical Research 2014; 3(10): 1404-1414.
19. Audisio M. Carina, María J. Torres, Daniela C. Sabaté, Carolina Ibarguren, María C. Apella Properties of different lactic acid bacteria isolated from Apis mellifera L. bee-gut. Microbiological Research 2011; 166(1):1–13.
20. Upendra R.S., Khandelwal P and Mohammed Ausim. A novel approach for enhancement of Lovastatin production using Aspergillus species. International Journal of Agriculture and Environmental Biotechnology 2013; 6:779-786.
21. Zhou X, Y.J. Pan, Y. BWang and w.F. Li Optimization of media composition for Nisin fermentation with Response Surface Methodology. Institute of Food Science 2008; 73(6): M245-9.
22. Zhao Ruixiang, Gaili Duan, Tianyou Yang, Shengyang Niu and Ying Wang. Purification,

Characterization and Antibacterial Mechanism of Bacteriocin from Lactobacillus Acidophilus XH1. Tropical Journal of Pharmaceutical Research 2015; 14 (6): 989-995.

23. Upendra R.S., Pratima Khandelwal. Assessment of nutritive values, phytochemical constituents and biotherapeutic potentials of Epiphyllum oxypetalum. International Journal of Pharmacy and Pharmaceutical Science 2012; 4(5), 421-425.
24. http://kb.psu.ac.th/psukb/bitstream/2553/1470/6/293920_ch2.pdf
25. Batdorj B., M. Dalgalarrondo , Y. Choiset , J. Pedroche, F. Me´tro , H. Pre´ vost, J.-M. Chobert and T. Haertle´. Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag. Journal of Applied Microbiology 2006; 101: 837–848.
26. Ravi Sankar, N. V. Deepthi Priyanka, P. Srinivas Reddy, P. Rajanikanth, V. Kiran Kumar and M. Indira. Purification and Characterization of Bacteriocin Produced by Lactobacillus plantarum Isolated from Cow Milk. International Journal of Microbiological Research 2012; 3 (2): 133-137.

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