Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIBACTERIAL ACTIVITY OF BACTERIOCIN PRODUCED FROM *LACTOBACILLUS* AND *LACTOCOCCUS*

ARUNAVA DAS*, M. E. SINDUJA, K. HARSHADHA, J. BINDHU, R. BALAKRISHNARAJA

Molecular Diagnostics and Bacterial Pathogenomics Research Laboratory, Department of Biotechnology, Bannari Amman Institute Technology, Sathyamangalam, Erode District, Tamil Nadu, India

ABSTRACT

Bacteriocins from bacteria inhibit the growth of similar or closely related bacterial strains. Present investigation was focused on to check the antibacterial activity of bacteriocin produced from *Lactobacillus* and *Lactococcus* bacteria against various pathogens of food spoilage and bovine mastitis. A total of 62 (15.93%) *Lactobacillus* and 22 (5.65%) *Lactococcus* isolates were tentatively identified from 389 commercial food products by using morphological, biochemical and molecular characterization. All the isolates were further confirmed has *L. plantarum* [22 (5.65%)] and *L. acidophilus* [18 (4.62%)] and *Lactococcus lactis* [22 (5.65%)] by polymerase chain reaction. In PCR, *recA* gene (318bp) and 16s-23s rRNA interspecific spacer regions (575bp) were amplified from the isolates of *L. plantarum* and *L. acidophilus* respectively, whereas, *gad*B (602bp) gene was amplified from *Lactococcus lactis*. The optimal conditions for the extraction and partial purification of bacteriocin were standardized by using ammonium sulphate precipitation and sephadex column chromatography respectively. Bactericidal activity of all bacteriocin producing *Lactobacillus* and *Lactococcus* isolates were tested against six major indicator bacteria of food spoilage and bovine mastitis like *Listeria monocytogenes, Escherichia coli, Salmonella enterica* Typhimurium, *Staphylococcus aureus, Streptococcus agalactiae* and *Staphylococcus epidermis*. The crude bacteriocin produced anaerobically by *L. acidophilus* isolates after 96hrs of incubation at 37°C showed higher antibacterial activity than *L. plantarum* and *Lactococcus lactis*. The purified bacteriocin showed significantly increase in the zone of inhibition against *Staphylococcus aureus* and *Streptococcus agalactiae* than other bacteria. Present investigation suggested that the PCR is a simple, rapid and reliable method of diagnosis of *Lactobacillus* and *Lactococcus* bacteria up to species level. The purified bacteriocin produced from *L. acidophilus* was found to have inhibitory action against the Gram positive and Gram negative pathogens in foodborne and bovine mastitis .

KEYWORDS: ***Lactobacillus acidophilus, Lactococcus lactis, Lactobacillus plantarum, Bacteriocin, Polymerase chain reaction.***

ARUNAVA DAS *

Molecular Diagnostics and Bacterial Pathogenomics Research Laboratory, Department of Biotechnology, Bannari Amman Institute Technology, Sathyamangalam, Erode District, Tamil Nadu, India

Received on: 31-08-2017
Revised and Accepted on: 05-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b432-439

Creative commons version 4.0

INTRODUCTION

The major concern in food industry is the food spoilage caused by the food borne pathogens. The consequences of quality loss of food product caused by microorganisms leads to food borne illness to both humanbeings and animals. The U.S. Food and Drug Administration (FDA) gives a high priority to protect people from hazards of microbial contamination. The FDA concerned food borne bacteria are listed in Bacteriological Analytical Manual, FDA[1]. These include *Escherichia coli, Salmonella, Shigella, Campylobacter, Yersinia enterocolitica, Y. pseudotuberculosis, Vibrio cholera, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens, C. botulinum* and *Cronobacter* species and many more. Bovine mastitis is another risk factor that leads to an inflammation in udder tissue of cows and is difficult to control in dairy industry. It is caused by various Gram positive and Gram negative bacterial species, of which *Staphylococcus* and *Streptococcus* are the commonest. The most widely used probiotics for human beings are Bifidobacteria and Lactobacilli. Lactic acid bacteria (LAB) are safe microorganisms that improve disturbances in the indigenous microflora, ameliorate the development of microflora[2]. Lactic acid bacteria are widely present in the nature as well as in the fermented food[3]. Bacteriocins are defined as small ribosomally synthesized, membrane permeabilizing, cationic antimicrobial peptides. Despite varying chemical structure, mode of action, and host specificity, any protein secreted by bacteria that has an inhibitory effect on other bacteria is classified as a bacteriocin. These bacteriocins have the potential to inhibit a narrow range of lactic acid bacteria, Gram positive bacteria, and food spoiling bacteria. Some investigators have reported on the ability of bacteriocins to also inhibit Gram-negative bacteria. Bacteriocins and the organisms that produce them have potential in the food and feed industry as natural preservatives, as well as in the pharmaceutical industry as a source of probiotics[4].Bacteriocins are potentially useful in food and feed industries because of their antibacterial characteristics; moreover, bacteriocins can be used as bio preservatives in fermented foods[5]. Nisin, Generally Recognized As Safe (GRAS) bacteriocin, produced by *Lactococcus lactis subsp. lactis* is the only bacteriocin currently approved by more than 50 countries for use in certain food industry applications [6,7,8]. LAB are usually known as safe (GRAS), and have an important role in the preservation of foods and fermented products. They can be used as natural competitive microbiota or as specific starter cultures under controlled conditions[9]. Some of these bacteria produce antagonistic substances, called bacteriocins, which in small amounts are very active against pathogens. Antagonistic properties of lactic acid bacteria (LAB) allied to their safe history of use in traditional food fermented products make them very attractive to be used as bio preservatives. The increasing interest in these compounds has stimulated the isolation of LAB producers and the characterization of many novel peptides[10].The successful development of Nisin from an initial biological observation through regulatory approval for commercial applications is a model that has stimulated new contributions in the field of bacteriocin research[11].In this view, the study on molecular diagnosis and antibacterial activity of bacteriocin produced from *Lactobacillus* and *Lactococcus* has been carried out that may lead to an effective remedy to solve the current problems of food spoilage and fatal disease like bovine mastitis. The present research is focused to extract and purify the bacteriocin produced by the lactic acid bacteria and to test their bactericidal activity against pathogens responsible for causing food spoilage and bovine mastitis[11].

MATERIALS AND METHODS

Sample collection

A total of 389 samples comprising of meat, fish products, milk and dairy products, raw vegetables, bakery products, beverage and fermented rice products were investigated in this study. All the samples were procured from randomly selected local retail shops, local markets and supermarkets in Salem, Erode, Tirupur, Namakkal and Coimbatore districts of Tamil Nadu, India and immediately transferred to the laboratory for isolation and microbiological analysis.

Indicator microorganisms

Foodborne pathogens and bovine mastitis pathogens like *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica* Typhimurium, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Staphylococcus epidermis* were procured from Microbial Type Culture Collection, Chandigarh, India and American Type Culture Collection, USA to study the antagonist properties of isolates.

Isolation of lactic acid bacteria

All the samples were aseptically inoculated in to Nutrient Broth (Hi-Media Laboratories, Mumbai), incubated aerobically at growth temperature of 37°C for 24hr. The samples from nutrient broth were inoculated into culture specific medium like De man Rogosa Sharpe (Hi-Media Laboratories) and were incubated aerobically at 37°C for 24hr. From the inoculated plates colonies showing different morphological characters were randomly selected, repeated subculture was carried out each time in fresh plates until pure culture was obtained. The pure cultures were grown in De man Rogosa Sharpe (MRS) agar plates and preserved at 4°C.Glycerol stocks were made and the pure cultures are preserved at Deep freezer (-80˚C).

Morphological, biochemical and phenotypical characterization

- Pure bacterial isolates grown in MRS agar were subjected to various morphological, biochemical and phenotypical characters as per the Bergey's manual of determinative bacteriology with little modification[12].These tests included, motility of the bacteria, Gram's and endospore staining, nitrate reduction, sugar fermentation, starch hydrolysis, Voges-Proskauer, citrate, hydrogen sulfide, methyl red, indole, hemolysis, catalase, oxidase, coagulase, alkaline dehydrogenase, gelatinase, esculinase, ornithine decarboxylase and urease test etc.

Scanning electron microscopy of intestine samples

A single bacterial colony from each lactic acid bacteria were subjected for SEM analysis. The SEM was done commercially from Karunya University, Coimbatore. The bacteria were observed, photographed and analyzed under the JEOL JSM-6360 (Tokiyo, Japan) scanning electron microscope.

PCR Assay

The polymerase chain reaction was carried out with all the isolates of Lactobacillus and Lactococcus using species specific primers of *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactococcus lactic subsp lactis* using standard PCR condition. The species specific primer pairs include LacidoF: 5/-cacttcggtgatgacgttgg-3/ and LacidoR: 5/-cgatgcagttcctcggttaagc-3/ targeting 575bp [13]planF: 5/-ccgtttatgcgg aacaccta-3/, and pREV: 5/-tcgggattaccaaacatcac-3/ targeting 318bp[14]and gadB21: 5/-cgttatggatttgatggatataaagc-3/ and gad7: 5/-actcttcttaagaacaagtttaacaga-3/ targeting 602bp [15] to amplify the *23SrRNA, recA* and *gadB* genes respectively were commercially synthesized (Eurofin Genomic India Ltd, Bangalore). The template DNA for PCR was prepared by boiling and centrifugation method. The amplification products were analyzed by electrophoresis on a 1.5 % agarose gel containing 0.5µg/ml of ethidium bromide and the bands were visualized.

Single step PCR for Lactobacillus acidophilus

The reaction mixture in 25µl contained 1 unit of *Taq* DNA polymerase, 2mM of $Mgcl_2$ and 0.2mM each of dNTP and concentration of template DNA was 12.5ng and primer was 1.7µM. Each PCR product was amplified by the following conditions: Initial denaturation step for 5 min at 95˚C, followed by 30 cycles of denaturation for 30 sec at 95˚C, annealing for 30 sec at 63˚C, and elongation for 30 sec at 72˚C. The final extension step was 5 min at 72˚C.[15].

Single step PCR for Lactobacillus plantarum

For PCR amplification 20 ng of template DNA was added to a 20µl PCR mixture containing 1 U/µl of Taq polymerase, 0.25mM of each dNTP and 10X buffer. The reaction mix with template DNA was cycled through the following temperature profile: 94˚C, 3 min; 94˚C, 30 sec; 46˚C, 10 sec; 72˚C, 10 sec. The PCR reaction was terminated at 75˚C for 10 min and thereafter cooled to 4˚C.

Single step PCR for Lactococcus lactis

The reaction mixture in 50µl contained 200 ng of template DNA, 20 pmol of each primer, reagent mixture and *Taq* polymerase. The PCR conditions were as follows: Initial denaturation step for 9 min at 94˚C, followed by 45 cycles of denaturation for 30 sec at 94˚C, annealing for 30 sec at 50˚C, and elongation for 60 sec at 72˚C with an additional extension step was 7 min at 72˚C after the last cycle.

Screening of isolates for bacteriocin production

The crude bacteriocin was produced by growing *L. acidophilus* (18 isolates), *L. Plantarum* (22 isolates) *and Lactococcus lactis subsp. lactis* (22 isolates) in MRS broth separately, incubated at 37 °C for different time intervals such as 48hr, 72hr, 96hr and 120hr anaerobically. A total of 6 different indicator bacteria were investigated to test the antagonistic activity initially. The ability of the isolates to produce high amount of bacteriocin was tested by performing well diffusion assay method[16].The culture was centrifuged at 10000×g for 5 min and the supernatant collected [13-15]. A volume of 1 mL of inoculum of each indicator bacteria (A=1.0 at 540 nm) was lawn cultured on nutrient agar plates using sterile cotton swab . Wells of 7 mm in diameter and 5 mm deep were cut in each plate and different volume such as 20, 30 and 40 µL of culture supernatant was added. The zone of inhibition were recorded for different indicator bacteria to select the best strains. From the result obtained, 4 isolates of *Lactobacillus acidophilus*, 3 isolates of *Lactobacillus plantarum* and 2 isolates of *Lactococcus lactis subsp lactis* showed bacteriocin activity. These isolates were further tested for antagonistic activity to find out a novel bacteriocin which is very effective. The methodology for the production of bacteriocin by Lactic acid bacteria were also optimized based on its effective antimicrobial activity against indicator organisms.

Extraction of bacteriocin

The MRS broth (250 mL) was seeded with a novel bacteriocin producing strain of *L. acidophilus* and incubated at 37°C anaerobically for 96 h. The bacterial suspension was centrifuged at 10000×g for 10 min and collected the supernatant. Precipitation of the protein was done by adding $(NH4)_2SO_4$ at 40 % of saturation level. The precipitated pellet was collected after centrifugation at 20000×g at 4°C for 10 min and dissolved in phosphate buffer (0.1 M, pH=7.0). The protein sample was dialyzed against the same buffer for 12hr and purified sample was stored at 4°C for further use.

Sephadex column chromatography

Sephadex G-100 (5 g) was weighed and suspended in 500 mL of phosphate buffer overnight according to the recommended procedure. It was swollen for 5 h in boiling water bath followed by de-aeration for 1 h and then cooled to room temperature before packing the column. The dimensions of the used column were 75×1.5 cm. The packing was done with a precaution to avoid entrapment of any air bubbles in the gel bed. The column was then washed with phosphate buffer (pH=7.0, 0.1 M). The sample (5 mL) was loaded on Sephadex G-100 column, then it was eluted with phosphate buffer (pH=7.0) and sample fractions (0.5 mL) were collected in micro centrifuge tubes. The flow rate 5 ml/min (150 cm/hour) of the sample was kept constant. The fractions were analyzed for the protein content by measuring the absorbance at 280 nm and for the bacteriocin activity by using the spot plate method. Higher rate of inhibition showing protein fraction was selected.

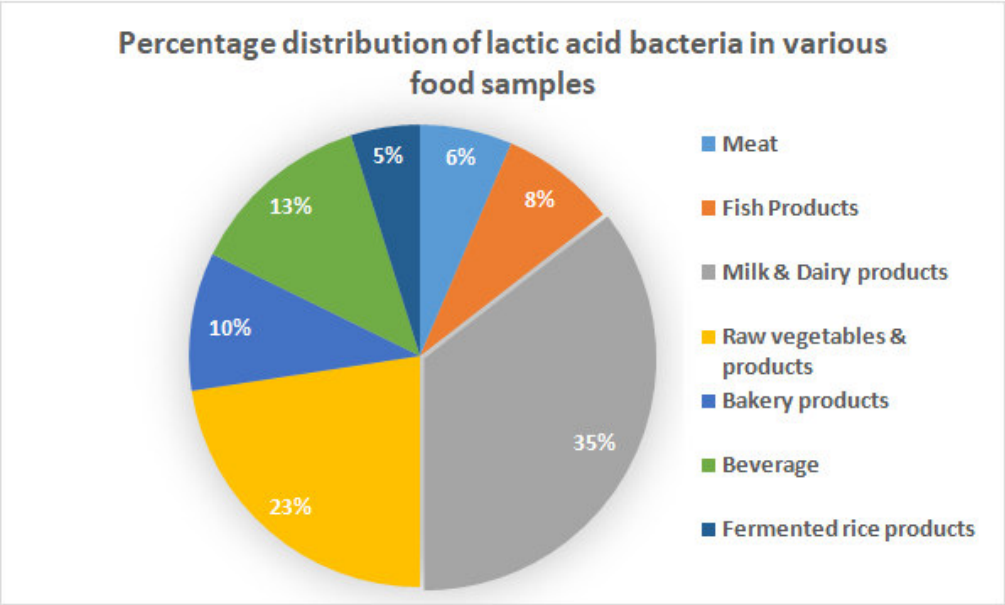
Antimicrobial assay

Anti-microbial property of the purified bacteriocin against various food and bovine mastitis pathogens was performed by well diffusion assay[17].The column purified bacteriocin sample was taken for assay. A volume of 1

mL of inoculum of each indicator bacteria (A=1.0 at 540 nm) was lawn cultured on nutrient agar plates using sterile cotton swab. Wells of 7 mm in diameter and 5 mm deep were cut in each plate and 30 µL of bacteriocin was poured. The plates were incubated at optimum temperatures for 24 hr and the clear zones formed around the wells were measured.

RESULTS AND DISCUSSION

Out of 389 dairy and fermented food products, 62 isolates of lactic acid bacteria were identified by analyzing various morphological, biochemical and phenotypical characteristics. The percentage distribution of these bacteria in different food samples is shown in Graph 1. In ultrastructure study, *Lactobacillus plantarum* and *L. acidophilus* were observed to be in clusters of thick rods. The rods were observed to be variable in length. The *Lactococcus lactis* was observed to be clusters of cocci, occasionally arranged in short chains formed by several bacteria (Fig 1). In PCR, *recA* gene (318bp) and 16s-23s rRNA interspecific spacer regions (575bp) were amplified from the isolates of *L. plantarum* and *L. acidophilus* respectively, whereas, *gad*B (602bp) gene was amplified from all *Lactococcus lactis*. PCR result revealed that out of 40 isolates of Lactobacillus, *L. plantarum* and *L. acidophilus* were confirmed in 22 (55%) and 18 (45%) numbers respectively, whereas, *Lactococcus* isolates were confirmed as *Lactococcus lactis subsp. lactis* in 22 (100%) numbers (Fig 2). Similar isolation and identification of food spoilage pathogens, bovine mastitis pathogens and bacteriocin producing bacteria from various dairy, food and fermented food products have been reported[18,19,20]. The screening of all the isolates of LAB for bacteriocin production showed only 4 isolates of *Lactobacillus acidophilus*, 3 isolates of *Lactobacillus plantarum* and 2 isolates of *Lactococcus subsp. lactis*, were inhibiting the growth of indicator organisms. A varied zone of inhibition was observed for different indicator organisms. From the results observed the hyper bacteriocin producing strain was found to be *L. acidophilus* which was selected and grown on nutrient agar plates. The crude bacteriocin was extracted from 96 hr grown *L. acidophilus* isolates by centrifugation at 10,000 rpm for 5 min. In this study, the formation of maximum zones of clearance upto 12 mm was observed against *Staphylococcus aureus* (Graph 2) and was also supported by other researchers[21]. Partial purification of bacteriocin was achieved by adding ammonium sulfate to the culture supernatant at 40 % level of saturation. The precipitate formed was dissolved in phosphate buffer and stored at 4°C. Partial purification of precipitate by Sephadex column chromatography resulted in a total of 10 protein fractions. The UV spectrophotometry revealed that out of 10 fractions of *L. acidophilus* only 5 fractions had high protein content. The well diffusion assay results showed only one fraction each with larger diameter of zone of inhibition of 16-18 mm produced against *Staphylococcus aureus* isolates. The purification of bacteriocin by Sephadex column chromatography has been performed[22]. The Antimicrobial assay results using partially purified bacteriocin revealed a remarkable increase in the diameter of zones of inhibition for micro-organisms like *S. aureus* (18mm), *S. agalactiae* (15mm). The maximum inhibition was observed for *S. aureus* (Graph 3). The comparison of inhibitory action of crude and purified bacteriocin revealed an increase in the potential of bacteriocin after purification (Graph 4). This change is due to the removal of interference of impurities and thus higher concentration of protein in the fraction. Bacteriocins have generally been reported to dissipate trans-membrane potential and increase the membrane permeability to ions, leading to the collapse of the proton motive force and thus killing the cell[23].Bactericidal mode of action causes the death of a pathogen, thus, it is capable of eradicating the major population of undesirable microorganisms from food items. The bacteriocin protein was found to have inhibitory action against Gram positive bacteria viz *S.aureus*[24].The findings of this study correlate with the findings of Bizani and Brandelli[25].The inhibition was found higher in isolates of gram positive origin. Bacteriocin from *L. acidophilus* is having bright prospects to be used as an effective food bio-preservative and as therapeutics, as it has strong antagonism against a broad range of challenging and commonest food spoilage and bovine mastitis pathogens. The study paves the way of exploring the possibilities of large scale production of bacteriocin from *L. acidophilus* to be used as an antibiotic against for the treatment of bovine mastitis in dairy and as a bio-preservative in various food stuffs. A budding expediency of bacteriocins justifying a more in profound research for their identification and application as food bio preservatives and therapeutics is thus revealed.



Graph 1
Percentage distribution of lactic acid bacteria in various food samples

Ultrastructure study of lactic acid bacteria by scanning electron microscopy

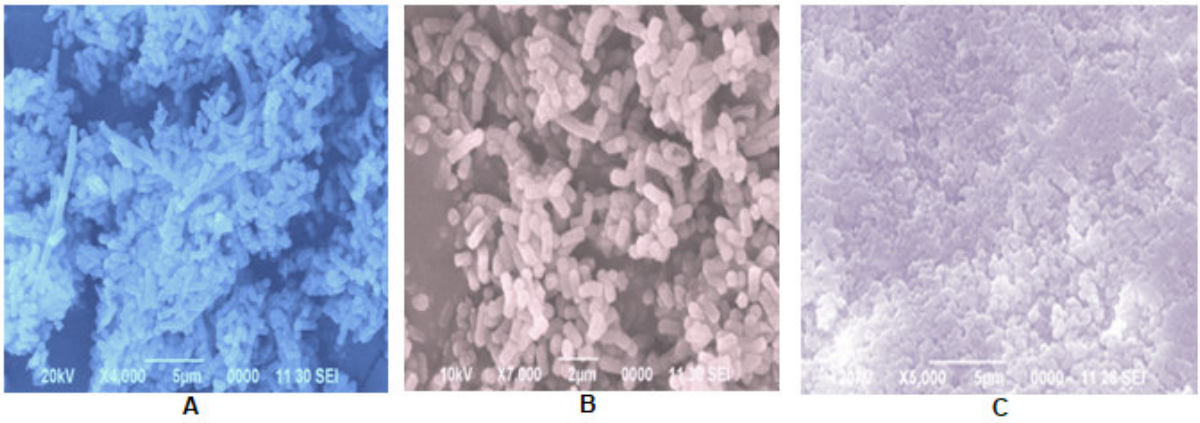


Figure 1
ultrastructure study of lactic acid bacteria under SEM showed L. plantarum (A), L. acidophilus (B) and Lactococcus subsp. Lactis (C)

Identification of isolates by polymerase chain reaction assay

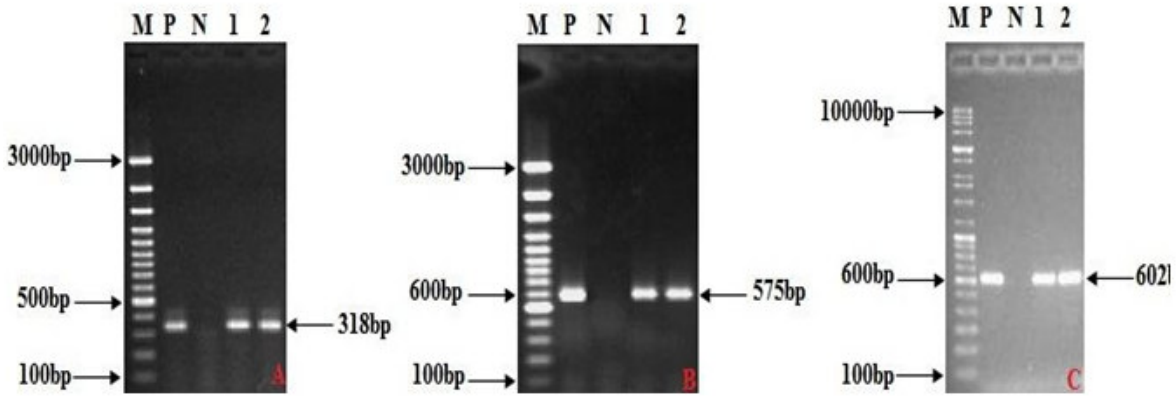
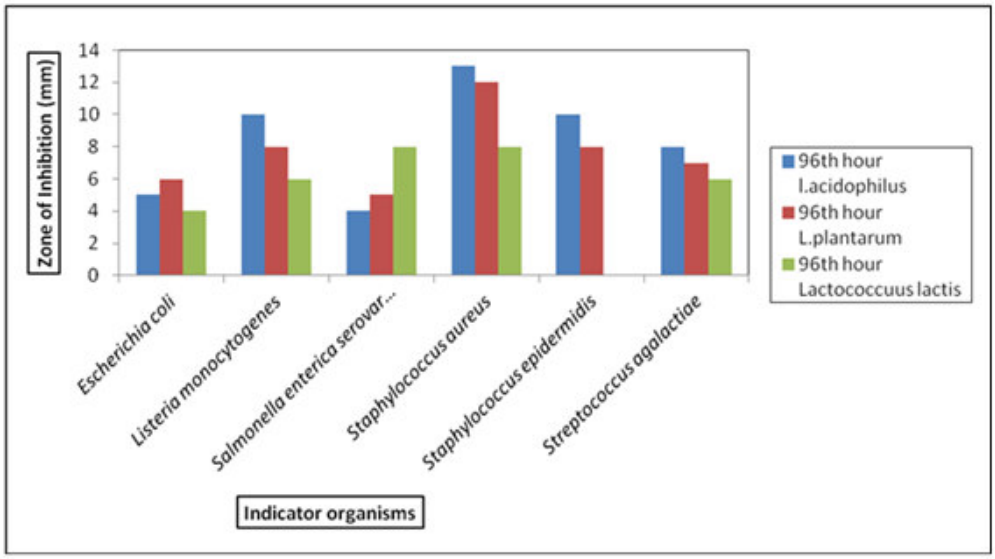
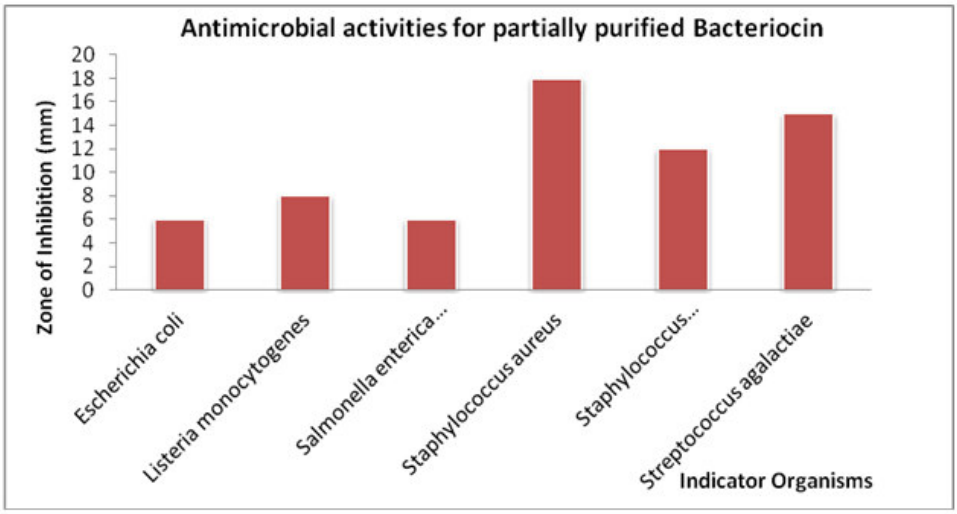


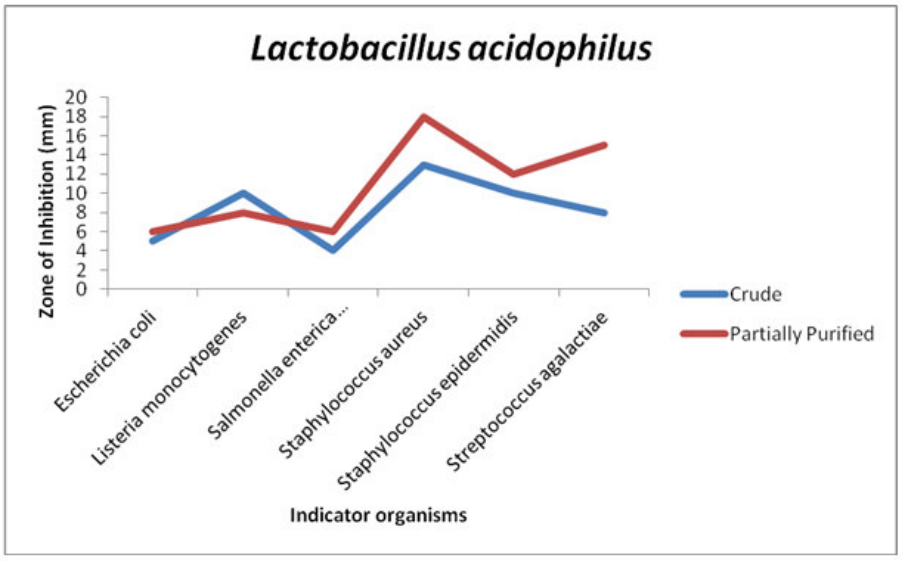
Figure 2
gene amplification by PCR showed A: recA gene of 318 bp; B: 23S rRNA gene of 575 bp; C: gad B of 602 bp of L. plantarum, L. acidophilus and Lactococcus subsp. lactis respectively. Lane M: 100bp DNA Ladder, Lane P: Positive control, Lane N: Negative control, Lane 1 & 2: Field isolates



Graph 2
Screening of isolates for bacteriocin production



Graph 3
Antimicrobial activities for partially purified bacteriocin



Graph 4
Comparison of crude and partially purified bacteriocin from Lactobacillus acidophilus

CONCLUSION

Present investigation suggested that the PCR is a simple, rapid and reliable method of diagnosis of *Lactobacillus* and *Lactococcus* bacteria up to species level. The purified bacteriocin produced from *L. acidophilus* was found to have inhibitory action against the indicator bacterial pathogens in foodborne and bovine mastitis.

FUNDING ACKNOWLEDGEMENT

The authors wish to acknowledge the financial grant vide reference no. 20/AICTE/RIFD/RPS (POLICY-1)28/2012-13 under Research Promotion Scheme of All India Council of Technical Education (AICTE), New Delhi to execute this research work as a part of the funded project. Authors would also like to convey thanks to Bannari Amman Educational Trust for the kind support.

CONFLICT OF INTEREST

Conflict of interest is none.

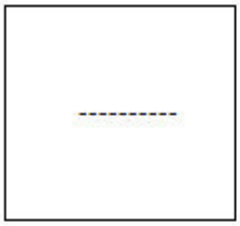
REFERENCES

1. Food and Drug Administration,Bacteriological Analytical Manual.7th and 8th ed. Arlington, VA: AOAC International.2012.
2. Prathiba S and Saravana KP.Heat Killed Probiotic Lactobacillus Plantarum Induces Nf Kappa B Factor Compared with live bacteria and Lipopolysaccharides on Hek – 293 Cell Lines International Journal of Pharm Bio Sciences, 2016; 7(3): 438 - 441.
3. Poonam B Chauhan1 and Divya Daru. Isolation and Characterization of Lactobacillus isolated from Milk, Curd and Fecal Sample and assigning their Probiotic values. International Journal of Pharma Bio Sciences, 2016 ; 7(3): 1070 – 75
4. Green DH, Wakeley PR, Page A, Barnes A, Baccigalui L,Ricca E,and Cuttin M. Characterization of two Bacillus Probiotics Applied Environmental Microbiology,1999;65:4288–91.
5. Ralph WJ,Tagg JR,and Ray B.Bacteriocins of gram-positive bacteria. Microbiology,1995;59:249–52.
6. Klaenhammer TR.Bacteriocins of lactic acid bacteria. Biochimie., 1988;70:337–49.
7. Stevens KA, Sheldon BW, Klapes NA, Klaenhammer TR. Nisin treatment for inactivation of *Salmonella* species and other Gram-negative bacteria. Applied and Environmental Microbiology, 1991;57:3613-15.
8. Delves BJ.Blackburn P,Evans RJ,and Hugeholtz J.Applications of the bacteriocin, nisin.Antonie Van Leeuwenhoek, 1996;69(2):193–202.
9. Cintas LM,Herranz C,Hernández PE,Casaus MP,and Nes LF.Review: Bacteriocins of lactic acid bacteria.Food Science andTechnology, 2001;7:281-305.
10. Deraz SF,Karlsson EN,Hedstrom M,Andersson MM,and Mattiasson B.Purification and characterisation of acidocin D20079, a bacteriocin produced by *Lactobacillus acidophilus* DSM 20079.Journal of Biotechnology, 2005;117:343-54.
11. Deegan LH,Cotter PD,Hill C,and Ross P.Bacteriocins: Biological tools for biopreservation and shelf-life extension.International Dairy Journal, 2006;16:1058-71.
12. Holt JG,Krige NR,Sneeth PHA,Staley JT and Williams ST.Bergey's Manual of Determinative Bacteriology.Ninth edition. Williams and Wilkins Baltimore, USA,1994;9:749-55.
13. Sul, Su-Yeon, Hyun-Joong Kim, Tae-Woon Kim and Hae-Yeong Kim.Rapid Identification of *Lactobacillus* and *Bifidobacterium* in Probiotic Products Using Multiplex PCR,Journal of Microbiology and Biotechnology, 2007;17(3):490-95.
14. Sandra Torriani, Giovanna E. Felis, and Franco Dellaglio: Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *rec*A gene sequence analysis and multiplex PCR assay with recA gene-derived primers. Applied and Environmental Microbiology.2001; 67(8).3450–54.
15. Massara MLA, Alves JB, Brandão PRG: Atraumatic restorative treatment: clinical. ultrastructural and chemical analysis. Caries Research 2002; 36: 430–36.
16. Aslim B, Yuksekdag ZN,Sarikaya E and Beyatli Y.Determination Of The Bacteriocin like Substances Produced by some Lactic Acid Bacteria Isolated from Turkish Dairy Products.Journal of Biology, Chemistry.2004;120:233-36.
17. Sharma N,Riti K,Neha G and Ranjana K.Purification of Bacteriocin by *Bacillus subtilis R75*.Food Technology Biotechnology, 2011;49(2):169–176.
18. Shome BR,Das S, Bhuvana M,Krithiga N,Velu D,Shome R,Isloor S,Barbuddhe SB,and Rahman, H .Multiplex PCR assay for species identification of bovine mastitis pathogens.Journ al of Applied Microbiology, 2004 ;111:1349–56.
19. Van Reenen CA,Dicks LMT,and Chikindas ML.Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*.Journal of Applied Microbiology,1998;84:1131–37.
20. Das A,Sreehari S,Shalini U,Ganeshkumar A and Karthikeyan M,Molecular screening of virulence genes from *Salmonella enterica* isolated from commercial food stuffs. Biosciences Biotechnology Research Asia, 2012;9(1):363-369.
21. Jack RW,Tagg JR,and Ray B.Bacteriocins of Gram-Positive Bacteria. Microbiology Review,1995; 59:171-200.
22. Anu C and Norman, J. Role of the Leader and Structural Regions of Prelantibiotic Peptides as Assessed by Expressing Nisin-Subtilin Chimeras in *Bacillus subtilis 168*, and Characterization of

their Physical, Chemical, and Antimicrobial Properties.Journal of Biology and Chemistry,2001;270:23533-539.

23. Montville TJ.and Chen Y.Mechanistic Action of Pediocin and Nisin: Recent Progress and Unresolved Questions.Applied Microbiology and Biotechnology,1998;50:267-274.
24. Amanda S,Florencia O and Adriano B.Screening for antimicrobial activity among bacteria isolated from Amazon basin. Brazilian Journal of Microbiology,2004;35(1)42-48.
25. Bizani D and Brandelli A.Characterization of a Lisboa and bacteriocin produced by a newly isolated *Bacillus* sp. strain 8. Advanced Journal of Applied Microbiology, 2002; 93:521-519.

Reviewers of this article



Dr. K. Balakrishnan, M.Sc., Ph.D.

Professor, Principal Scientific Officer,
BIT-TBI, Bannari Amman Institute of Technology,
Sathiyamangalam -638401, Erode Dist,
Tamilnadu, India.

Dr.Kanakam Elizabeth Thomas, M.Phil, Ph.D.

Assistant Professor, Dept. of Microbiology,
SRM Dental College, Ramapuram, Chennai
600089, India.

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript