Production and characterization of a bacteriocin produced by Enterococcus faecium LR/6

M Kumar, N Ghosh, S Srivastava

Citation

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Abstract

A soil isolate of Lactic acid bacteria (LAB), strain LR/6 was identified as Enterococcus faecium on the basis of its morphological, biochemical, and 16S rDNA analysis. Strain LR/6 is a potential producer of an antimicrobial compound that followed the kinetic pattern typical of a primary metabolite synthesis and is released extracellularly. The antimicrobial action remained unaffected when treated with β-glycerophosphate and catalase suggesting that the activity is neither based on acid nor H_2O_2 production. This antimicrobial compound was highly stable over a wide range of pH (2.0 to 8.0), high temperature (boiling and autoclaving) and could be stored stably at a range of temperature (-20ºC to 37ºC) at least upto one year. The proteinaceous nature of the antimicrobial compound was ascertained by its sensitivity to many proteolytic enzymes confirming it to be a bacteriocin. Strain LR/6 bacteriocin was also insensitive to the presence of α-amylase, lipase, surfactants, organic solvents, and showed a bactericidal mode of action. Crude bacteriocin exhibited broad inhibition spectrum both against related as well as some food-borne pathogenic bacteria including Listeria monocytogenes. Tricine SDS-PAGE analysis and bacteriocin activity assay corresponded to a protein of an apparent molecular mass of ~6.0 kDa.

INTRODUCTION

Among Lactic acid bacteria, members of the genus Enterococcus are widely distributed throughout nature as inhabitants of the gastrointestinal tract of human and other animals and are also found in vegetables, plant materials and foods (Giraffa, 2002; Koehler, 2007). Bacteria belonging to these genera are recognized by their bacteriocinogenic activity against Listeria monocytogens and in recent years several new enterocins have been reported (Foulquie-Moreno et al., 2006; Bruna et al., 2008).

Lactic acid bacteria have been used in the production of a variety of dairy, vegetables, meat and fermented foods from many centuries. In addition to the contribution to the typical sensory characteristics of these foods (Fox and Wallace, 1997), LAB exerts strong antagonistic activity against many food-contaminating microorganisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins (Piard and Desmazeaud, 1992). Bacteriocins produced by LAB are of great interest to the food fermentation industry as natural preservatives because of their ability to inhibit the growth of many food spoilage and pathogenic bacteria including L. monocytogens, Staphylococcus aureus, Bacillus cereus and Clostridium botulinum (Bredholt et al., 2001).

The bacteriocins of LAB have been classified into four classes on the basis of common, mainly structural, characteristics (Klaenhammer, 1993). Most of the bacteriocins isolated so far belong to classes I or II. Class II bacteriocins have emerged in recent years as the most promising bacteriocin candidates for food preservation, as they display overall better performance, in terms of biological activity and physico-chemical properties, than most bacteriocins from other classes (Nes and Holo, 2000, Garneau et al., 2002).

This paper reports the identification of a bacteriocin producing strain LR/6 by phenotypic, biochemical and genetic method. In addition, production conditions, physico-chemical properties, and mode of action of the crude bacteriocin are described.

MATERIALS AND METHODS

BACTERIAL STRAINS, CULTURE MEDIA, GROWTH CONDITIONS AND CHEMICALS

Strain LR/6 isolated from soil was routinely propagated in normal TGYE medium (5 $g.L^{-1}$ tryptone, 1 $g.L^{-1}$ glucose, 3 $g.L^{-1}$ yeast extract, and pH 7.0) medium at 37ºC and 200 rpm in an incubator shaker (Kuhner, Switzerland) as described earlier (Tiwari and Srivastava, 2008). For the purpose of

production of bacteriocin, an optimized TGYE medium (20 $g.L^{-1}$ glucose, 20 $g.L^{-1}$ yeast extract, 15 $g.L^{-1}$ tryptone, 1.0 $g.L^{-1}$ tween 80, 3.0 $g.L^{-1}$ triammonium citrate, 11.3 $g.L^{-1}$ sodium acetate, 3.0 $g.L^{-1}$ K_2HPO_4, 0.5 $g.L^{-1}$ $MgSO_4$, 0.2 $g.L^{-1}$ $MnSO_4$, pH 7.0) at 37°C and 200 rpm. Micrococcus luteus used as an indicator organism was grown in nutrient broth (5 $g.L^{-1}$ peptone, 3 $g.L^{-1}$ beef extract, 5 $g.L^{-1}$ NaCl, and pH 7.0) under similar conditions. Growth was generally monitored turbidometrically in terms of optical density (A_{630}) (Genesys 10vis, Thermospectronic, USA). The related LR strains were obtained from our own collection and other strains of lactic acid bacteria were from Northern Regional Research Laboratory (NRRL), Agricultural Research Service Culture Collection, USA. The pathogenic strains from Department of Microbiology, University of Delhi, South Campus, New Delhi, and Department of Virology, AIIMS, New Delhi, India, were used to observe the host range of bacteriocin of strain LR/6. All the chemicals were obtained from Sigma-Aldrich, USA and media components were purchased from Hi-media, Mumbai, India.

PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF THE STRAIN

Cells of strain LR/6 were examined by light microscopy to determine their morphology and tested for Gram-staining reaction and catalase activity. Other identification tests included, the ability to grow at 4, 10 and 45ºC, on media containing 1 to 10% NaCl, growth at different pH (4.4 to 10.0), on different carbon sources and hydrolysis of arginine (Table 1). Carbohydrate utilization profiling was also done by API 50CHL system (BioMerieus, Lyon, France).

For the identification based on 16S rDNA sequence. DNA from the strain was isolated from 1.5 mL of log phase culture using Bacterial Genomic DNA kit (Sigma-Aldrich, USA). By using the set of universal primers pA (5' AGA GTT TGA TCC TGG CTC AG 3') and pE (5' CCG TCA ATT CCT TTG AGT TT 3') (Beasley and Saris, 2004), the region of the 16S rRNA gene was amplified by 29 cycles of PCR (consisting of 30 s at 94ºC, 60 s at 55ºC, and 90 s at 72ºC, with a final 120 s extension step at 72ºC) with purified chromosomal DNA from strain as template. The amplification assay comprised ~50 ng of template DNA, along with master mix that included reaction buffer, dNTPs, magnesium chloride and Taq DNA polymerase (BIOTOOLS, Spain), and 25 pmol of each oligonucleotide primers in a final volume of 25 µL. PCR assay was carried out on a GeneAmp^R PCR system 2700 (Applied Biosystems, USA). The amplified 900-bp fragment was resolved on 1% horizontal agarose gel, purified by means of the Gel elution kit (mdi, India), and sequenced using an automated gene sequencer (ABI PRISM 310). The sequence so obtained was aligned and compared with known sequences of 16S rRNA gene of different lactic acid bacteria strains from the database using BLAST (www.ncbi.nlm.nih.gov. /BLAST/).

Further confirmation of the result was done using species-specific primers of Enterococcus faecium 16S rRNA gene from NCBI, database. These consisted of EntFm(F) 5'-TGCAAGTCGTACGCTTCTTTT-3' and EntFm(R) 5'-CAATCATCTATCCCACCTTAGGC-3'. Amplification conditions were as follows (2 min at 94ºC, 1 min at 92ºC, 1.5 min at 50ºC, 2 min at 72ºC, 5 min at 72ºC).

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The 16 S rDNA sequence of strain LR/6 so obtained was submitted to GeneBank (NCBI) under the Accession number EU366176.

GROWTH AND BACTERIOCIN PRODUCTION

An overnight grown culture of strain LR/6 (~10^6 $CFU.mL^{-1}$) was used to inoculate the optimized TGYE medium and was incubated at 37ºC and 200 rpm over a period of 24 h. Growth was measured periodically in terms of A_{630} and viable cells were counted by plating an appropriate dilution on TGYE agar plates. Bacteriocin production was quantified in terms of $AU.mL^{-1}$ by microtitre plate assay method as described later. The change in pH was also recorded.

PREPARATION OF BACTERIOCIN SAMPLE

Strain LR/6 cells grown in optimized TGYE medium at 37ºC, 200 rpm for 18 h were harvested by centrifugation at 12000g for 10 min at room temperature and the supernatant was collected. The supernatant was filtered through 0.2 µm membrane (mdi, India) and quantified in terms of activity unit per ml ($AU.mL^{-1}$) in a microtitre plate.

BACTERIOCIN ACTIVITY ASSAY

The antimicrobial activity of the bacteriocin was routinely determined by the agar-well diffusion assay (AWDA) method (Tagg et al., 1976). Aliquots of supernatant (100 µL) were placed in wells (6 mm diameter) cut out in cooled TGYE agar plate, overlaid with soft agar (5 mL) seeded with a target strain (□10^6 $CFU.mL^{-1}$). The plate was incubated overnight at 37ºC and halo produced, if any, was used as an indicator of growth inhibition.

Bacteriocin activity was quantified by a microtitre plate

assay (Holo et al., 1991). For this, the bacteriocin sample was serially diluted two-fold in 50 µL volumes of nutrient broth in a 96-well microtitre plate. Each well was filled with 200 µL nutrient broth containing the indicator strain (~10^6 $CFU.mL^{-1}$) derived from a fresh overnight culture. The plate was incubated for 6 h at 37ºC and growth was measured as A_{630} using a Microplate Reader (Bio-Rad, USA). One bacteriocin unit was defined as the amount of bacteriocin, which inhibited the growth of the indicator organism by 50% as compared to the untreated control.

Effect of -glycerophosohate and catalase treatment

In order to neutralize the effect of lactic acid, the crude bacteriocin was treated with sodium -glycerophosphate (1.7% final concentration). The mixture was incubated at 37ºC for 2 h and antimicrobial activity was determined. Similarly, one sample was treated with catalase (final concentration 5 $mg.mL^{-1}$) so as to see involvement of hydrogen peroxide.

PHYSICO-CHEMICAL PROPERTIES OF CRUDE BACTERIOCIN

Crude bacteriocin was treated to a range of pH, temperature and storage at different temperatures so as to see the effect of the same on its activity. To check the pH response, the crude bacteriocin was adjusted to different pH ranging from 2.0 to 8.0 in 1: 1 ratio of different buffer solutions (HCl-KCl 50 mM, pH 2.0 and 4.0; phosphate buffer 50 mM pH 6.0, 7.0 and 8.0) and incubated for 4 h at 37ºC. Then the samples were sterilized by filtration through 0.2 µm membrane and antimicrobial activity was determined in terms of $AU.mL^{-1}$. Negative controls consisted of buffers of different pH used, and untreated crude bacteriocin was used as positive control.

To evaluate the heat stability the crude bacteriocin was incubated at 60, 90 and 100ºC for 30 min and one sample was autoclaved (121ºC, 15 psi, 15 min). The effect of extended storage at different temperature 37ºC, room temperature (ranging from 10 to 30 ºC), 4ºC and (-) 20ºC was assessed by regularly monitoring its activity upto one year. A positive control, consisting of freshly prepared crude bacteriocin was tested each time in parallel.

SENSITIVITY TO HYDROLYTIC ENZYMES, SURFACTANTS AND ORGANIC SOLVENTS

To demonstrate the proteinaceous nature of the antimicrobial compound, it was treated with different proteolytic enzymes: proteinase K, pepsin, papain, -chymotrypsin, and protease (Sigma-Aldrich, USA) at a final concentration of $1mg.mL^{-1}$, and incubated at 37ºC for 2 h. The crude bacteriocins in buffer without enzymes as well as the enzymes in buffer solutions were used as control. The samples were tested for antimicrobial activity as described above. The crude bacteriocin was also treated with -amylase and lipase, each at $1mg.mL^{-1}$ final concentration, to show its effect on the activity.

The surfactants used were SDS, Tween 80, Tween 20, Triton X-100 and urea, which were added to the crude bacteriocin at a final concentration of 1% (v/v) and incubated at 37ºC for 5 h. Surfactants at 1% in TGYE broth were used as controls. Activity in all samples was determined as above. Surfactants (Sigma, USA) were prepared as 10% aqueous solution and filter sterilized before use.

The crude bacteriocin was mixed with various organic solvents (ethanol, methanol, isopropanol, acetone, ethyl acetate and toluene) at a final concentration of 50% (v/v). After incubation for 2 h at 37ºC, the organic solvents were evaporated and residual antimicrobial activity was determined as described earlier.

MODE OF ACTION

To determine the mode of action, the indicator strain was grown to an exponential stage (~10^6) then resuspended in normal saline (0.85%) and examined for the inhibition with 200 $AU.mL^{-1}$ of crude bacteriocin. Samples were withdrawn periodically, and plated on NB-agar medium for determination of $CFU.mL^{-1}$. Untreated cells served as control.

DETERMINATION OF ANTIMICROBIAL SPECTRUM

To determine the antimicrobial spectrum a wide range of bacteria comprising some LAB and food-borne pathogens were tested against the crude bacteriocin. Effect was ascertained both by $CFU.mL^{-1}$ and agar well diffusion assay (AWDA).

TRICINE SDS-PAGE

Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed, as a step gradient gel (4, 10, and 16.5% acrylamide for the stacking, spacer and separating gel, respectively), according to the procedure of Schägger and Von Jagow (1987). Myoglobin fragments (16,950, 14,440, 10,600, 8160, 3480 and 2510 Da) were used as marker proteins (Sigma, USA). Samples were run in duplicate. One part of gel containing the molecular mass markers and sample was stained with Coomassie Brilliant

Blue R-250 (CBB-R-250), to visualize the protein. The other lane, containing only sample, was extensively washed with regular changes of sterile MilliQ water for overnight. For direct detection of antimicrobial activity, the gel was overlayed with soft agar (0.8%) seeded with indicator strain M. luteus followed by overnight incubation at 37ºC (Bhunia et al., 1987; De Vuyst et al., 1996).

STATISTICAL ANALYSIS

Each result is expressed as mean along with respective Standard error of mean. Each data point is the average of three repeated measurements from two independent replicates.

RESULTS AND DISCUSSION

Strain LR/6 was identified to be Gram-positive, coccoid in shape, and catalase-negative. The strain was able to grow between 30ºC to 45ºC, in a pH range of 4.4-10.0 even in the presence of 1 to 6.5% NaCl, and produced ammonia from arginine (Table 1).

The carbohydrate utilization pattern determined by API 50CHL analysis showed that strain LR/6 could utilize a large number of sugars except xylose and two sugar alcohols

(Table 1). Based on the observed characteristics, the isolate was tentatively classified as belonging to the genus Enterococcus. In addition, the identification of strain was also carried out using 16S rDNA sequencing. The rRNA gene amplified from strain LR/6 using universal primers comprised ~900 bp. This amplicon was sequenced and when the sequence was blasted (www.ncbi.nlm.nih.gov./BLAST/) against all the known 16S rDNA sequences of lactic acid bacteria, it exhibited highest similarity with known sequences of Enterococcus faecium. Further, phylogenetic analysis showed that strain LR/6 clustered in E. faecium group showing 99% similarity with 16S rRNA gene sequence of E. faecium DO ctg 623, DO ctg 654, and DOc ctg 565 (Figure 1A). Identification was further confirmed by PCR amplification, using species-specific 16S rRNA gene primers from conserved part of E. faecium showing an amplicon of ~1.5 kb (Figure 1B). The strain LR/6 could then be identified as Enterococcus faecium.

Figure 1

Table 1: General identification and characterization of strain LR/6

Test	**Result**
Gram's stain	Positive
Morphology	Coccus
Catalase	Negative
Arginine hydrolysis	+
Growth at	
4ºC	-
10ºC	-
30ºC	+
37ºC	+
45ºC	+
pH 4.4	+
pH 5.0	+
pH 6.0	+
pH 7.0	+
pH 8.0	+
pH 9.6	+
pH 10.0	+
NaCl 1%	+
NaCl 3%	+
NaCl 5%	+
NaCl 6.5%	+
NaCl 10%	-
Arabinose	+
Cellobiose	+
Fructose	+
Galactose	+
Glucose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Sucrose	+
Xylose	-
Sorbitol	-
Glycerol	-

(+) = Growth; (-) = No growth

Figure 2

Figure 1A: Phylogenetic dendogram derived from the analysis of ~900bp amplicon from strain LR/6 to show the evolutionary relationship of strain LR/6.

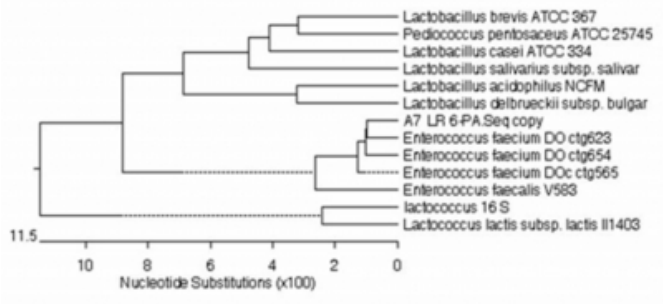
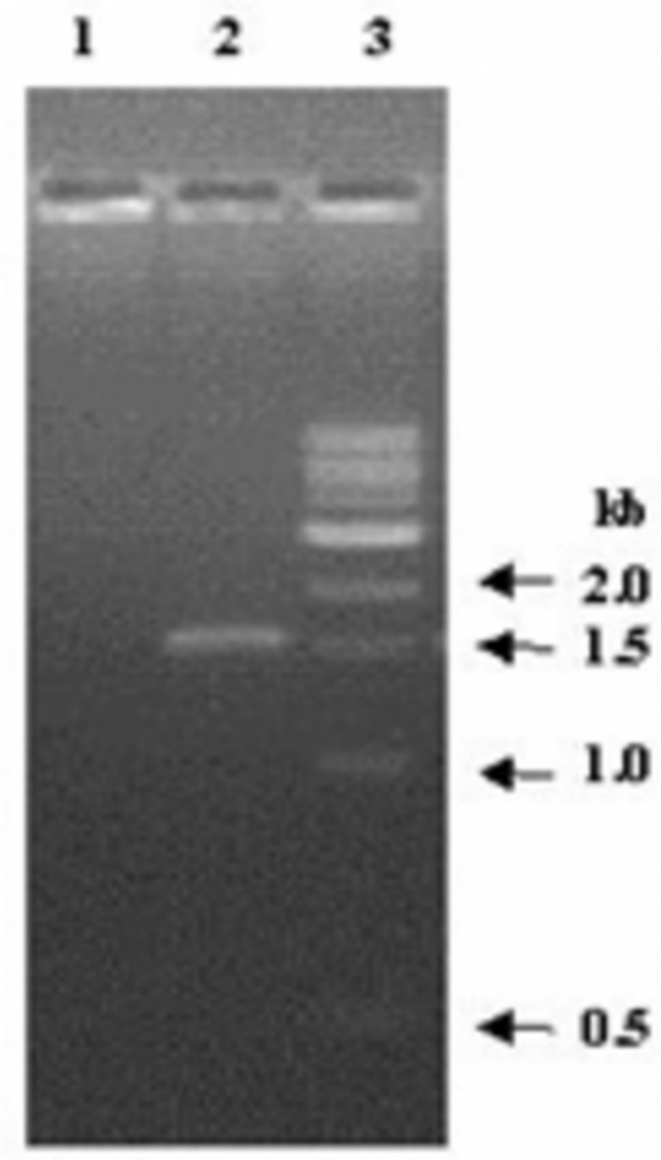


Figure 3

Figure 1B: Figure 1.5: PCR product with primers from conserved sequence of 16S rRNA gene. Lane 1: LR/14; Lane 2: Strain LR/6; Lane 3: DNA ladder (1 kb).

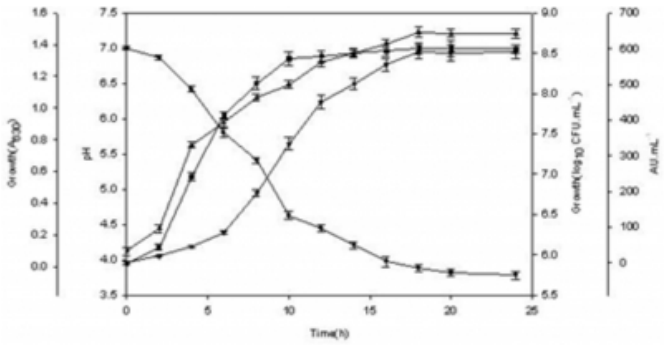


Strain LR/6 when grown in optimized TGYE medium at 37ºC, showed a typical sigmoidal growth response and associated bacteriocin production (Figure 2) Antimicrobial activity could be detected in the culture filtrate after 4 h of incubation, (the mid-log phase) with maximum accumulation in stationary phase (18 h). Thereafter, the bacteriocin activity remained constant till 24 h. The pH of the medium was dropped from neutral to 3.8 during this period. The antimicrobial activity of the culture supernatant was not affected by treatment with □-glycerophosphate or catalase suggesting that neither acid nor H_2O_2 production are responsible for the same. Alternatively, it may also indicate that E. faecium LR/6 produces a peptide antibiotic or

bacteriocin. The kinetic pattern of production appeared to be typical of a primary metabolite, as suggested by several authors before (Ferreira et al., 2007; Ghrairi et al., 2008). Lacticin NK 24 followed the same pattern but it drastically dropped after the late stationary phase (Lee and Paik 2001), and bacteriocin produced by P. pentosaceus K23-2 (Shin et al., 2008).

Figure 4

Figure 2: Growth of strain LR/6 in optimized TGYE medium (□), change in pH (□), log CFU.mL (□) and bacteriocin production (□).



The crude bacteriocin of strain LR/6 was stable over a wide range of pH (2.0-8.0). The maximum activity was obtained between pH 2.0 to 6.0, and a loss of ~20%, was observed at pH 7.0 and 8.0. Of the other bacteriocins isolated from Enterococcus, A5-11 bacteriocin (Parente and Hill, 1992) was found to be active over a pH range from 2.0 to 10.0 (Batdorj et al., 2006). The inhibitory activity also remained unaffected by heating at 60, 90, and 100ºC for 30 min and even autoclaving (at 121ºC, 15 psi for 15 min). Heat and pH stability could be considered a very useful characteristic as many of these bacteriocins may find a potential use in food preservation. Similar types of results have been reported for other bacteriocins (John et al., 2009; Simova et al., 2009). The notable exceptions are lactocin NK 24 that lost 87.5% of its activity at boiling temperature and completely inactivated upon autoclaving (Lee and Paik, 2001), and bacteriocin N15 produced by E. faecium N15, which is stable at 100ºC but is completely inactivated by autoclaving (Losteinkit et al., 2001). The antimicrobial activity of crude bacteriocin was also found intact even after storage (one year) at different temperatures as described in Material and Methods, a result which is in agreement with the enterocins produced from E. faecium (Audisio et al., 2005; Simonova and Laukova, 2007).

To determine the chemical nature of the antimicrobial compound, the effect of some proteolytic enzymes (proteinase K, pepsin, papain, □-chymotrypsin and protease) was studied. The active compound was found to be sensitive to these enzymes, but □-amylase, lipase did not affect, indicating that the inhibitory material is proteinaceous in nature. Similar results have been reported for the bacteriocins from Enterococcus (Ghrairi et al., 2008; John et al., 2009). While the plantaricin TF711 was completely and partially inactivated by □-amylase and lipase, respectively (Hernandez et al., 2005), bacteriocins from L. mesenteroides L124 and L. curvatus L442 were inactivated by lipase (Mataragas et al., 2002). The crude bacteriocin of strain LR/6 remained stable even when treated with surfactants like SDS, Tween 80, Tween 20, Triton X-100 and urea. Similar results have been reported for a bacteriocin from E. mundtii ST 15 (De Kwaadsteniet et al., 2005), planatricin C19, from L. plantarum (Atrih et al., 2001) and enterocin EJ97 produced by E. faecalis EJ97 (Galvez et al., 1998). In contrast, inactivation has been reported of plantaricin TF711 from L. plantarum TF711 (Hernandez et al., 2005) and bacteriocin produced by L. delbrueckii 1043 (Miteva et al., 1998) after such treatments. Similar stability was observed even after treatment with organic solvents, as also reported for lacticin NK 24 (Lee and Paik, 2001), and plantaricin (Navarro et al., 2000).

The effect of crude bacteriocin on the cell viability of indicator strain, M. luteus was traced in 0.85% NaCl, in comparison to an untreated control. As shown in Figure 3, the antimicrobial action was so effective that within 2 h, the population was reduced from 6.11 $\log_{10}$ CFU.mL^{-1} to 3.39 $\log_{10}$ CFU.mL^{-1} and after 6 h no viable cells were observed. This loss in viability showed bactericidal mode of action. The mechanism of this inhibition is under investigation. A similar mode of action has been observed for many other bacteriocins from lactic acid bacteria (Bendali et al., 2008; Papagianni and Anastasiadou, 2009). In contrast, many other bacteriocins showed bacteriostatic effects (Harnandez et al., 2005; Todorov and Dicks, 2005).

Figure 3: Bactericidal effect of crude bacteriocin on M. luteus cells. The results are shown as ~6.1 $\log_{10}$ CFU.mL^{-1} after incubation with 200AU/mL^{-1}of bacteriocin (□) and without (□) for different incubation times.

In order to study the antimicrobial host range, several LAB strains and food-borne pathogenic bacteria comprising both Gram-positive and Gram-negative members were tested. As is clear from the results highly efficient activity was observed against L. monocytogenes, Pseudomonas

aeruginosa , Aeromonas sp., Yersinia enterocolytica,

Figure 5

Table 2: Inhibitory spectrum of crude bacteriocin produced by strain LR/6

Test organism	Media	Sensitivity
LAB isolates		
LR strains LR/3, 4, 5, 10, 16	TGYE	+
LR strains LR/1, 14	TGYE	-
Enterococcus casseliflavus NRRL B-3502	MRS	+
Lactococcus lactis lactis NRRL B-1821	MRS	+
Lactobacillus pentosus NRRL B-227	MRS	-
L. acidophilus NRRL B-4495	MRS	-
L. acidophilus NRRL B-3468	MRS	-
L. plantarum NRRL B-4496	MRS	-
L. helveticus NRRL B-4526	MRS	+
L. casei casei NRRL B-1922	MRS	-
L. delbrueckii NRRL B-1924	MRS	-
L.lactis cremoris NRRL B-634	MRS	-
L. brevis NRRL B-4527	MRS	+
L. mesenteroides mesenteroides NRRL B-1118	MRS	-
L.vitulinus NRRL B-14854	MRS	-
L.sakei NRRL B-1917	MRS	-
L.delbrueckii NRRL B-763	MRS	-
Micrococcus luteus MTCC 106 (indicator strain)	NB	+
E. coli K-12 MTCC 1302	LB	+
Food Born Pathogens		
Yersinia enterocolytica	TSB	+
Aeromonas sp.	NB	+
Listeria monocytogens	NB	+
Pseudomonas aeruginosa	NB	+
Shigella sp.	NB	+
E. coli (urogenic)	LB	-
Staphylococcus aureus	NB	-
Salmonella typhimurium	NB	-
Bacillus sp.	NB	-
Bacillus licheniformis	NB	+
Vibrio cholera	NB	-

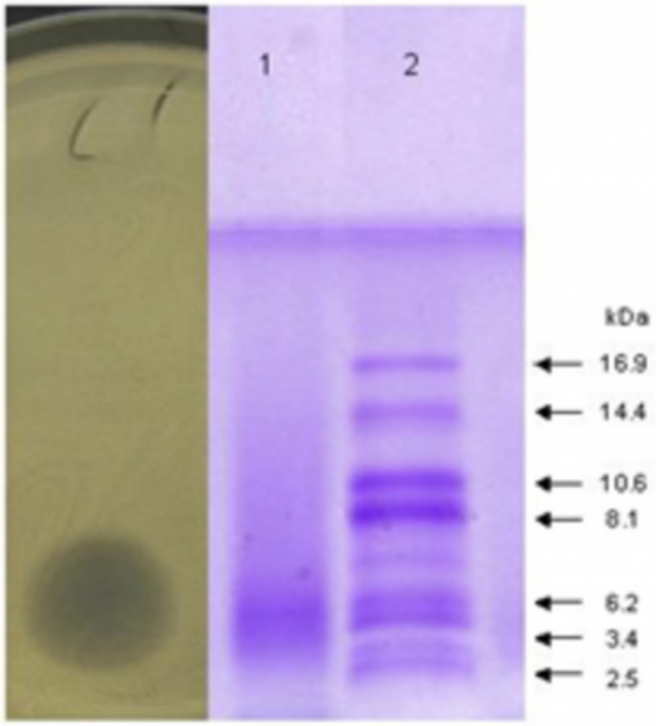
(+) = sensitive; (-) = resistant

Bacillus licheniformis, Shigella sp., E. coli. K-12 as well as also against several strains of LAB (Table 2). Enterocins from several species of Enterococcus have been reported to exhibited similar type of response such as E50-52 produced from E. faecium E50-52 (Edward et al., 2008), enterocin P-like from E. faecium GM-1 (Kang and Lee, 2005). However, enterocin IT from E. faecium IT62 showed very narrow antimicrobial spectrum (Esther et al., 2008). The majority of the enterocins belong to class II, which are active against Gram-positive bacteria, especially listerial species (Casaus et al. 1997; Audisio et al., 2005).

As described in Material and Methods, the crude bacteriocin of strain LR/6 was resolved on the SDS-PAGE. The electrophoretogram of the gel stained with Coomassie Brilliant Blue R-250 showed a diffused band of protein corresponding to a molecular mass of ~6.0 kDa (Figure 4). When the gel was overlaid with the indicator bacteria a large inhibitory zone corresponding to this band could be observed. The molecular size of LR/6 bacteriocin is close to that produced by E. avium (Audisio et. al., 2005). On the otherhand, enterocin ON-157 from E. faecium NIAI 157 is one of the lowest molecular weight bacteriocin (Ohmomo et al., 2000).

Figure 6

Figure 4: Tricine-SDS-PAGE of crude bacteriocin. Lane 1: position of peptide band with corresponding zone of inhibition. The gel was overlaid with (~10 CFU. mL), imbedded in TGYE soft agar. Incubation was at 37 Â°C for overnight. Lane 2: molecular weight marker.



CONCLUSION

Enterococcus faecium LR/6 is a natural isolate and produced a bacteriocin. Strain LR/6 showed a typical sigmoidal growth response and the bacteriocin production followed the kinetic pattern typical of primary metabolite synthesis. The bacteriocin produced from strain LR/6 showed strong antimicrobial activity against most challenging and serious food born pathogens, like L. monocytogenes. The characterization of bacteriocin showed high thermal and pH stability, as also long-term storage stability (one year) at different temperatures. Its sensitivity to proteolytic enzymes suggested proteinaceous nature, with no carbohydrate and lipid moiety required for its activity. The bactericidal ~6.0 kDa size protein is also stable against surfactants and organic solvents. The unique combination of all these properties identified a new bacteriocin from Enterococcus faecium LR/6, which could find diverse applications including as an useful food biopreservative. Attempts are on to purify this protein so as to characterize it further.

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Author Information

Manoj Kumar, M. Sc.
Department of Genetics, University of Delhi South Campus

Nitika Ghosh, M. Sc.
Department of Genetics, University of Delhi South Campus

Sheela Srivastava, Ph. D.
Department of Genetics, University of Delhi South Campus