Isolation of anti-listerial bacteriocin producing Lactococcus lactis CFR-B3 from Beans (Phaseolus vulgaris)

P Kiran-kumar, V Badarinath, P Halami

Citation

P Kiran-kumar, V Badarinath, P Halami. *Isolation of anti-listerial bacteriocin producing Lactococcus lactis CFR-B3 from Beans (Phaseolus vulgaris)*. The Internet Journal of Microbiology. 2008 Volume 6 Number 1.

Abstract

Contamination of food samples by Listeria
monocytogenes is a major cause of food-borne infections. Controlling the growth of Listeria sp. by application of bacteriocin or bacteriocin producing-organism in foods is one of the challenges in the development of effective bio-preservation process. The objective of this study was to isolate and to characterize bacteriocinogenic strain Lactococcus lactis CFR-B3 from Indian beans. Bacterial strain of Pediococcus acidilactici NRRL B1153 was used as indicator to screen bacteriocin production. The culture was found to produce class IIa heat-stable anti-listerial bacteriocin of MW ~ 5 kDa. The use of food-grade organism as an indicator for screening bacteriocinogenic strains of lactic acid bacteria circumvent the need for using pathogenic cultures such as listeria spp. The native isolate Lc. lactis CFR-B3 finds its application in the preservation of vegetables due to its prevalence in the same ecological niche.

Source of fund: This work was financially supported from the institute project under MLP-0304

INTRODUCTION

Consumption of raw and fresh vegetables is known to be an essential aspect of all healthy food habits. Several culinary vegetables being consumed are known to be nutritious in the human diet. However, the possible spread of pathogenic bacteria in these foods has not viewed seriously. Listeriosis, caused by List monocytogenes, is a major food borne infection in humans. In the United State, as per the report by the Centre for Disease control, an estimated 2, 500 persons become seriously ill with listeriosis every year. Of them atleast 20% cases lead to fatality. One of potential source of pathogenic outbreak is contaminated food, fresh vegetables etc. Several reports indicated that different serotypes of List. monocytogenes transmit via dairy products (Cheese), meat samples and raw vegetables. As Listeria sp have the ability to grow at low temperature, cold storage of vegetables act as selective advantage for this species (Farber and Peterkin, 1991, Cotter et al., 2005; Resenquist et al., 2005). Incorporation of bacteriocin or bacteriocin producing lactic acid bacteria (LAB) in food has advantage in preservation by inhibiting the growth of food- borne pathogens like List. monocytogenes (Drider et al., 2006). A recent survey conducted jointly by the UK Health Protection Agency and local authority body LACORS uncovered two products with listeria levels above the EU's regulatory limit. About 5 per cent of mixed raw vegetable salads in the UK were found to be contaminated with Listeria monocytogenes (Little et al., 2007).

Indian beans, apart from its sole use in Indian curry, is being used for the preparation of several culinary dishes that are being served during normal meals. These include salad, vegetable soup, vegetable sandwitch, vegetable rolls, etc. Beside normal practices of minimal processing of the beans by MAP, the pathogenic bacteria can multiply during storage and transport. In order to ensure safety during raw consumption and to minimize the spread of pathogenic bacteria, biopreservation practices can be adopted that include the use of bacteriocinogenic cultures of LAB isolated from the same ecological niche as protective cultures (Kelly et al., 1998; Cotter et al., 2005; Pounce et al., 2007). Among LAB, Lactococcus lactis represent one of the important species, widely known for the production of nisin. Nisin is a ribosomally synthesized antimicrobial peptide, approved by FDA to be used for the preservation of canned foods and dairy products (Delves-Broughton et al., 1996). However, several reports indicated that this bacterium also

produces other novel, class IIa, non-lanthionine group of bacteriocin (Lee et al., 1999; Ghrairi et al., 2005). The objective of this study was to characterize the bacteriocin producing culture of Lactococcus lactis isolated from beans using classical and molecular biology techniques and partial characterization of bacteriocin.

MATERIAL AND METHODS

ISOLATION OF BACTERIOCIN PRODUCING STRAINS

Indian beans, also known as Green beans (Phaseolus vulgaris), were used as a source for the isolation of bacteriocinogenic LAB. The fresh young beans were washed, cut into small pieces and incubated in sterile 5% NaCl solution at room temperature to enrich resident microflora. Samples drawn at different time intervals were pour-plated on MRS agar (Hi Media, Mumbai; India). After sufficient growth, the plates having well separated colonies were overlaid with indicator bacterium Pediococcus acidilactici NRRL B1153 or Listeria monocytogenes Scott-A and allowed to incubate further. Colonies of LAB obtained from bean-fermentation that exhibited a zone of inhibition (>10 mm diameter) against the lawn of the indicator bacterium were picked aseptically and subjected for further studies.

ANTIMICROBIAL ACTIVITY ASSAY

Antimicrobial compound produced in the culture filtrate (CF) was characterized by agar well diffusion assay as described by Geis et al., (1983). One of the potent cultures identified based on its wider zone of inhibition (> 10 mm diameter) against the indicator B1153, was B3. It was subsequently used to study the antimicrobial spectrum against the series of indicator bacteria. Characterization of the antimicrobial compound was carried out as described by Halami et al. (2005).

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE ISOLATE

The isolate B3 was subjected for taxonomic identification as suggested previously (Lee et al., 1999); and as described in Bergey's manual of Systematic Bacteriology (Mundt, 1986). The 16S rRNA gene was PCR amplified as demonstrated previously (Halami et al., 2005) following standard techniques of molecular biology (Sambrook and Russell, 2001). Nucleotide sequences of the PCR product was determined and deposited in GenBank (Acc No. EU439292).

ANTIBIOTIC SENSITIVITY ASSAY

Antibiotic sensitivity pattern of the culture B3 was studied using an antibiotic sensitivity disc (Hi Media). Plasmid profiling of the test culture was performed by the method described by Anderson and McKay (Anderson and Mckay, 1983).

BACTERIOCIN CHARACTERIZATION

Antimicrobial compound produced by culture B3 was concentrated by extraction with chloroform following the method of Burianek and Yousuf (2000). Bacteriocin preparation was subsequently analyzed by Tricine SDS-PAGE (16.5T & 6C acrylamide) as described by Schagger and von Jagow (1987). Direct detection of antimicrobial compound was carried out as described by Bhunia et al. (1994)

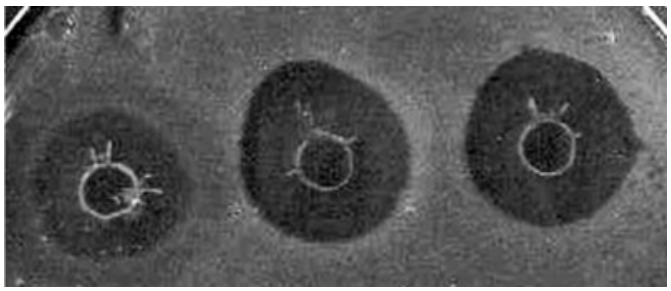
RESULTS AND DISCUSSION

ISOLATION OF POTENT BACTERIOCIN PRODUCING LAB

As many as 10 different bean samples procured from the market of the city of Mysore were subjected to screening for bacteriocinogenic cultures. The strain B3 was one of the potent cultures obtained among atleast 200 zone producing cultures against the indicator bacteria Ped. acidilactici B1153. One of the representative plates was also overlaid with List. monocytogenes Scott-A. The number of zone producers observed were almost same when two parallel plates of the same sample was overlaid with B1153 and Scott-A respectively. Hence, we successfully replaced the use of pathogenic strain Scott-A by B1153 as indicator for isolation of anti-listerial bacteriocin producers. Use of lactic culture as a bacteriocin indicator eliminated the effect of zone production due to acids, which is usually seen against pathogenic strains. The culture B3 was selected for further studies based on its antimicrobial activity in comparison to pediocin PA-1, and standard nisin preparations (Fig 1).

Figure 1

Figure 1: Comparative CF activity of native bean isolate against indicator B1153



1, CF of Ped. acidilactici K7 (Halami et al., 2005); 2, Lc. lactis CFR-B3 and 3, standard nisin preparation (1 IU/ml) obtained from Aplin and Barret (UK).

ANTIMICROBIAL SPECTRUM AND PROPERTIES OF ANTIMICROBIAL COMPOUND

Antimicrobial spectrum indicated that the CF of B3 was potent against the species of pathogenic bacteria such as Listeria and Bacillus as well as spoilage bacteria like Leuconostoc sp and Enterococcus sp. The antimicrobial compound was able to get inactivated by proteolytic enzymes and was stable at 100oC for 10 min. However, activity was lost upon autoclaving at 121oC for 20 min. The antimicrobial compound was active in a wide rang of pH (2 to 10). These results suggest that the antimicrobial compound is very similar to class IIa bacteriocins that have the properties of anti-listerial activity, heat-stability and activity over a wide pH range.

IDENTIFICATION OF BACTERIOCIN PRODUCING STRAIN

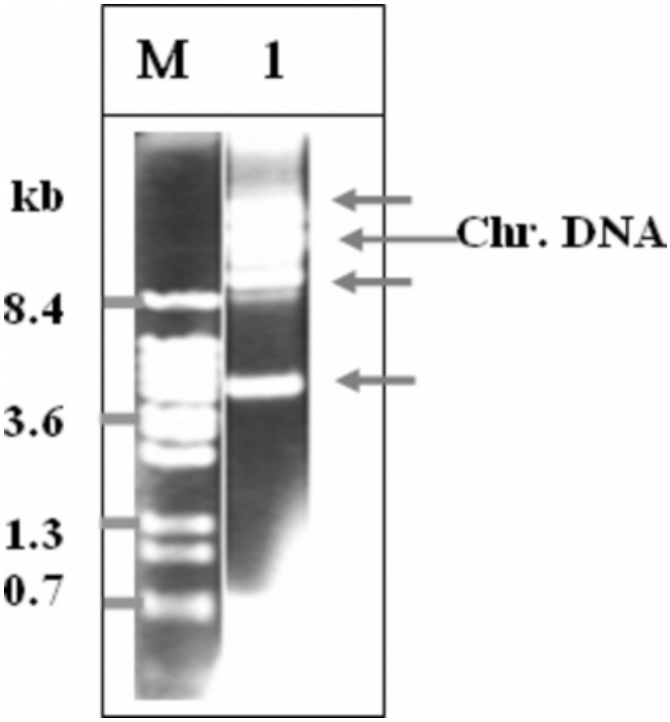
The culture B3 was unable to utilize citrate and was able to produce ammonia from arginine. Negative reaction for starch and gelatin hydrolysis were also observed. Optimum growth at 32oC, pH 6.5 as well as NaCl concentration of upto 4% was observed. The culture was able to utilize several plant sugars such as xylose, arabinose, sucrose etc. BLASTn search of the 16S rRNA gene sequences (582 bp) suggested >99% sequence homology with Lactococcus lactis (Acc no. EF589778), followed by several other species of Lactococcus lactis of vegetable and dairy origin including the genome sequence of Lc. lactis IL1403. With the microbiological characteristics of B3, in combination with 16S rRNA gene sequence data, we identified the native isolate as Lactococcus lactis CFR-B3, and deposited in the culture collection of this dept.

PLASMID PROFILING AND TRICINE GEL ANALYSIS

The plasmid profile of CFR-B3 indicated that it harbors at least one low molecular weight (MW) and two high MW plasmids (Fig 2).

Figure 2

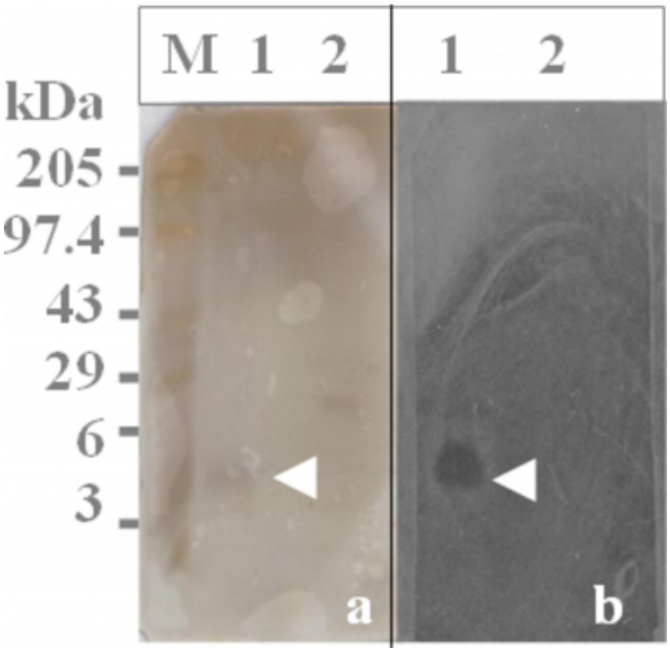
Figure 2: Agarose (0.8%) gel analysis of plasmid DNA isolated from CFR-B 3, lane 1. Arrow indicates different native plasmids and position of the chromosomal DNA band), M, ? DNA 911 digest (MBI, Fermentas).



One of the high MW plasmid resolved above the chromosomal DNA. The size of the smaller plasmid was approximately 7 kb. The plasmids found in CFR-B3 act as a marker in identification and are probably involved in bacteriocin production and carbohydrate fermentation. Tricine gel analysis.

Figure 3

Figure 3: Bioassay of bacteriocin preparation by Tricine SDS-PAGE. a, silver staining and b, activity assay overlaid with indicator B1153. lane 1, chloroform extracted sample and 2, trypsin treated chloroform extract. M, protein mol wt marker, medium range (Bangalore Genei, India). Arrow indicates zone producing protein bands.



revealed that bacteriocin of ~5 kDa exhibiting zone of inhibition against the indicator. Bacteriocin activity was abolished upon treatment with trypsin (Fig 3, lane 2b).

ANTIBIOTIC SENSITIVITY PATTERN

Antibiotic sensitivity test (Table 1) suggested that the strain was sensitive to a majority of antibiotics. That includes netilmycin, ciprofloxacin, lomefloaxcin and cefuroxime that characteristically deferred from Ped. acidilactici K7, a previously characterized pediocin PA-1 producer isolated from cucumber. Antibiotic sensitivity data suggested a desirable level of antibiogram that is generally acceptable for food applications.

Figure 4

Table 1: Comparative antibiotic sensitivity pattern of B3 and K7.

Antibiotic/s	Conc. (µg)	Culture/s K 7	Response* B 3
Amikacin	30	R	R
Ampicillin	10	S	R
Cefazoline	30	R	R
Chloramphenicol	30	S	S
Ceftriaxone	30	S	S
Ciprofloxacin	5	R	S
Cefroperazone	75	S	S
Ceptazidime	30	R	R
Cefotaxime	30	R	R
Cefadoroxil	30	R	R
Cefuroxime	30	R	S
Erythromycin	15	S	S
Gentamicin	10	R	R
Piperacillin	100	R	R
Roxythromycin	15	S	S
Penicillin	10#	R	R
Netilmicin	30	R	S
Norfloxacin	10	R	S
Nitrofurantoin	30	S	S
Lomefloxacin	10	R	S

Bacterial cultures used, K7; Pediococcus acidilactici K7 and B3,

Lactococcus lactis CFR B3. *R: Resistant; S: Sensitive; #unit concentration used

CONCLUSION

Well characterized cultures with bacteriocinogenic properties isolated from its natural ecological niche finds applications in food industry as a protective culture for Indian culinary purposes to reduce the risk of potential pathogenic bacteria associated with disease-outbreak as well as involved in natural spread of antibiotic resistance.

References

r-0. Anderson, D.G. and L. L. McKay. 1983. Simple and rapid method for isolating large plasmid DNA from Lactic streptococci. Appl. Environ. Microbiol. 46: 549 – 522.
r-1. Bhunia, A.K., Johnson, M.C. and Ray, B. 1987. Direct detection of an antimicrobial peptide of Pediococcus acidilactici in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. J Indust Microbiol 2, 319–322.
r-2. Burianek LL and Yousef AE. 2000. Solvent extraction of bacteriocins from liquid cultures. Lett Appl Microbiol. 31: 193-197.
r-3. Cotter, P.D., C. Hill and R.P. Ross. 2005. Bacteriocins: developing innate immunity for food. Nature Rev. Microbiol. 3: 777-788.
r-4. Delves-Broughton, J, P. Blackburn, R.J. Evans and J, Hugenholtz. 1996. Applications of the bacteriocin nisin. Ant. Van Leeuwenh. 69: 193-202.
r-5. Drider, D., Fimland, G., Hechard, Y., McMullen, L.M. and Prevost, H. 2006. The Continuing Story of Class IIa Bacteriocins. Microbiol. Mol. biol. Rev. 70(2). 564-582.
r-6. Farber, J.M. and Peterkin, P.I. 1991. Listeria monocytogenes, a Food-Borne Pathogen. Microbiological reviews, 55(3): 476-511.
r-7. Geis, A, J, Singh and M.J. Teuber. 1983. Potential of Lactic streptococci to produce bacteriocin. Appl. Environ. Microbiol. 45: 205-211.
r-8. Ghrairi T, J. Frere, J.M. Berjeaud and M. Manai. 2005. Lactococcin MMT24, a novel two-peptide bacteriocin produced by Lactococcus lactis isolated from rigouta cheese. Int. J. Food Microbiol. 105: 389-398.
r-9. Halami, P.M., A, Ramesh and A. Chandrashekar. 2005. Fermenting cucumber, a potential source for the isolation of pediocin-type bacteriocin producers. World. J. Microbiol. Biotechnol. 21:1351-1358.
r-10. Kelly, W.J., G.P., Davey and L.J.H. Ward. 1998. Characterization of lactococci isolated from minimally processed fresh fruit and vegetables. Int. J. Food Microbiol. 45: 85-92.
r-11. Lee, H-J, Y-J, Joo, C-S, Park, S-H, Kim, I-K, Hwang, J-S, Ahn and T-I. Mheen. 1999. Purification and characterization of a bacteriocin produced by Lactococcus lactis subsp. lactis H-559 isolated from kimchi. J. Biosci. Bioeng. 88: 153-159.
r-12. Little, CL, FC, Taylor, SK, Sagoo, I.A, Gillespie, K, Grant and J. McLauchlin. 2007.Prevalence and level of Listeria
monocytogenes and other Listeria species in retail pre-packaged mixed vegetable salads in the UK. Food Microbiol. 24: 7-8.
r-13. Mundt, J.O. (1986) Lactic acid streptococci. In: Sneath PHA, et al (Ed.), Bergey's manual of Systematic Bacteriology, Vol. 2. Williams & Wilkins, Baltimore, MD, pp. 1065-1066.
r-14. Pounce, AG, MR, Moreira, CE, Valle and S.I. Roura. 2007. Preliminary characterization of bacteriocin-like substances from lactic acid bacteria isolated from organic leafy vegetables. LWT – Food Sci. Technol. 41: 432-441.
r-15. Rosenquist, H., L, Smidt, S.R, Andersen, G.B, Jensen and A. Wilcks. 2005. Occurrence and significance of Bacillus cereus and Bacillus thuringiensis in ready-to-eat food. FEMS Microbiol. Lett. 250: 129-136.
r-16. Sambrook, J. and D.W. Russell. (2001). Molecular cloning: A laboratory manual (3rd Ed.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
r-17. Schagger, H. and von Jagow, G. 1987. Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analyt Biochem 166, 368– 379.

Author Information

P.M. Kiran-kumar, M.Sc.
Department of Food Microbiology, Central Food Technological Research Institute

V. Badarinath, M.Sc.
Department of Food Microbiology, Central Food Technological Research Institute

Prakash M. Halami, M.Sc., Ph.D.
Department of Food Microbiology, Central Food Technological Research Institute