Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Membrane-acting bacteriocin purified from a soil isolate *Pediococcus pentosaceus* LB44 shows broad host-range

Ramanjeet Kaur, Santosh Kumar Tiwari*

Department of Genetics, Maharshi Dayanand University, Rohtak, 124001, Haryana, India

ARTICLE INFO

Article history:
Received 26 February 2018
Accepted 8 March 2018
Available online xxx

Keywords:
Pediococcus pentosaceus LB44
Bacteriocin LB44
Antimicrobial activity
Mode of action
FTIR spectroscopy

ABSTRACT

Bacteriocin LB44 was purified from cell-free supernatant (CFS) of *Pediococcus pentosaceus* LB44 using activity-guided chromatography techniques. It was stable up to 121 °C, pH 2.0–6.0, sensitive to proteinase K, papain and trypsin, and retained complete activity in the presence of organic solvents tested. The molecular weight of bacteriocin was ~6 kDa and initial ten amino acid residues (GECGMCXECG) suggested a new compound. The loss in viable cell count and K^+ ion efflux of target cells of *Micrococcus luteus* suggested bactericidal activity. The cell membrane of bacteriocin-treated cells was found to be ruptured which was further confirmed by Fourier Transform Infrared (FTIR) analysis suggesting interaction of bacteriocin with phospholipids in cell membrane. It showed broad host-range and inhibited the growth of *Lactobacillus delbrueckii* NRRL B-4525, *L. plantarum* NRRL B-4496, *L. acidophilus* NRRL B-4495, *Enterococcus hirae* LD3, *Weissella confusa* LM85, *Staphylococcus aureus, Salmonella typhi* ATCC 13311, *Serratia marcescens* ATCC 27137, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 29905, *Haloferax larsenii* HA1, HA3, HA8, HA9 and HA10. These properties suggested a new bacteriocin from soil isolate *P. pentosaceus* LB44 which may offers possible applications in food-safety and therapeutics.

1. Introduction

Bacteriocins are extracellular secretory peptides and/or proteins demonstrating antimicrobial activity against related strains. Although, several bacteria have been reported to produce bacteriocins but lactic acid bacteria (LAB) are of particular interest due to their Generally Recognized as Safe (GRAS) status [1,2]. In addition, they have attractive characteristics such as heat tolerance, active over broad pH range, colourless, odourless and tasteless [3]. Nisin, produced by different strains of *Lactococcus lactis* is thoroughly studied bacteriocin used as a food biopreservative. However, it has limited applications due to lower antimicrobial activity in complex food matrix, reduced solubility at wide pH range and narrow host-range [4,5].

Bacteriocins produced by *Pediococcus* sp. are generally small, cationic, hydrophobic, heat and pH stable and sensitive to proteolytic enzymes [6,7]. They belong to Class II family of bacteriocins and contain N-terminal conserved motif 'YGNGVXC'. They show bactericidal mode of action against sensitive cells by pore-formation and release of intracellular contents showing broader antimicrobial spectrum against food-borne pathogens [1,8]. Various food isolates of bacteriocin-producing *P. pentosaceus* have been studied [1,7,9], however, bacteriocin-producing soil isolates of *P. pentosaceus* are not reported, to the best of our knowledge. Replacement of clinical antibiotics and chemical preservatives with natural and safe bacteriocins offer logical explanation for exploring new bacteriocins for their applications in therapeutics as well as food industries [10]. We have previously reported the isolation and characterization of *P. pentosaceus* LB44 from dairy soil for the presence of bacteriocin-like inhibitory substances (BLIS) in cell-free supernatant [11]. In present study, bacteriocin LB44 has been purified to homogeneity and characterized for stability, amino acid sequence, mode of action and host-range.

2. Materials and methods

2.1. Microbial strains and growth conditions

MRS broth medium [12] was used for growth of *P. pentosaceus* LB44 and production of bacteriocin. *M. luteus* MTCC 106 was grown in Nutrient Broth (NB) (g/l, peptic digest of animal tissue 5, NaCl 5, beef extract 1.5, yeast extract 1.5, pH 7.0). Reference LAB strains

* Corresponding author.
E-mail address: santoshgenetics@gmail.com (S.K. Tiwari).

https://doi.org/10.1016/j.bbrc.2018.03.062
0006-291X/

were obtained from Northern Regional Research Laboratory (NRRL), ARS Culture Collection, USA. Pathogenic strains were gift from Post Graduate Institute of Medical Sciences, Rohtak, Haryana. Other LAB and haloarchaea strains were available in our laboratory culture collection. *Escherichia coli* was grown in Luria Bertani and haloarchaea strains in AS-169 medium [13]. All the media components, enzymes and chemicals used in this study were procured from HiMedia, Sisco Research Laboratories, India and Sigma-Aldrich, USA.

2.2. Purification of bacteriocin

Bacteriocin from CFS of *P. pentosaceus* LB44 was purified as described previously by Gupta et al. [14]. Solid ammonium sulphate (w/v) was slowly added in 1 L CFS at final saturation levels 0–25, 25–50 and 50–80% and stirred continuously using magnetic stirrer at 4 °C. Protein pellets were collected by centrifugation at 8000 rpm for 15 min at 4 °C and solubilised in 10 mM sodium acetate buffer, pH 4.5 (Buffer I). The desalting was done by dialyzing against the same buffer using 2.0 kDa cut-off dialysis membrane (Sigma, USA) with several changes. The desalted sample was filter-sterilized through 0.2 μm filter (Axiva, India) and labeled as Fraction I. Cation-exchange chromatography (CEC) was performed using HiPrep SP FF 16/10 (1.6 × 10 cm, 20 ml) fitted with ÄKTAprime plus system (GE Healthcare, Sweden). The column was equilibrated with Buffer I at flow rate 1 ml/min. Fraction I was loaded on the column at flow rate 0.5 ml/min. The column was washed with 3x bed volume of Buffer I to remove unbound proteins. The elution of bound proteins was performed with a linear gradient of 0–1 M NaCl in Buffer I. The fractions (1.0 ml) were collected throughout the run. The flow rate was maintained at 1 ml/min and the protein elution profile was monitored at 214 nm. The antimicrobial activity of each fraction was determined in terms of AU/ml as described by Kaur and Tiwari (11). The active fractions were pooled and concentrated using lyophilization (Fraction II).

The gel-filtration chromatography (GFC) was performed using sephadex G-50 column (1.6 × 50 cm, 100 ml) (GE Healthcare, Sweden) fitted with ÄKTAprime plus system. The column was equilibrated with Buffer I containing 0.15 M NaCl. Fraction II (1 ml) was loaded on to column and elution was performed with the same buffer at flow rate 0.2 ml/min. The elution profile was observed at 214 nm and conductivity was recorded in terms of millisiemens per centimeter (mS/cm). The fractions (1 ml) were collected and antimicrobial activity of each fraction was determined as described above. The active fractions were pooled, concentrated using 3 kDa cut-off vivaspin (GE Healthcare, Sweden) and labeled as Fraction III. To test the homogeneity of purified sample, Fraction III was loaded on reverse phase C18 column (250 × 4.6 mm, PC18 HQ105, 5 μm) fitted with HPLC system (Youngling, South Korea). The column was equilibrated with 0.1% TFA, elution was performed using 60% acetonitrile containing 0.1% TFA at flow rate 1 ml/min and absorbance was monitored at 280 nm.

2.3. Tricine SDS-PAGE, bioassay and N-terminal sequencing

Tricine SDS-PAGE was performed as described by Schagger [15]. Purified bacteriocin was treated with loading dye (5x) at 37 °C for 15 min, centrifuged at 8000 rpm for 5 min and loaded in duplicate along with molecular weight marker (Bangalore Genei, India). Electrophoresis was carried out in miniVE vertical electrophoresis system (Amersham Biosciences, UK) at 30 V for stacking gel and 70 V for separating gel till the dye-front migrated at the end of the gel. One half of the gel was cut and used for staining while the other half was processed for bioassay. The gel was placed in fixing solution (methanol 50% and glacial acetic acid 10%) for 15–30 min and stained overnight in staining solution (methanol 50%, Coomassie Brillient Blue R-250 0.025% and glacial acetic acid 10%) for protein visualization. For bioassay, other half of gel was fixed in fixing solution and washed with sterile distilled water with frequent changes. The gel was placed on nutrient agar medium and overlaid with soft agar seeded with ~10^6 cells of freshly grown indicator strain, *M. luteus*. The plate was incubated at 37 °C for overnight and zone of growth inhibition was observed corresponding to protein band as described by Tiwari and Srivastava [16].

For N-terminal amino acid sequencing, western blotting of purified bacteriocin was performed as described by Schagger [15]. The required area of PVDF membrane containing bacteriocin was sent for N-terminal sequencing using Edman degradation method from Iowa State University, USA. The obtained sequence was analysed using online protein Basic Local Alignment Search Tool (blastp).

2.4. Stability of bacteriocin

The stability of bacteriocin LB44 was determined as described by Tiwari and Srivastava [16]. For heat stability, it was treated at 60, 80 and 100 °C in water bath and 121 °C under 15 psi pressure for 15 min. For pH stability, the sample was suspended in 1:1 ratio of different buffers ranging from pH 2.0–10.0 (50 mM HCl-KCl for pH 2.0 and 4.0, 50 mM phosphate buffer for pH 6.0, 50 mM tris-HCl for pH 8.0 and 10.0) and incubated for 2 h at 37 °C. The buffers of respective pH were taken as control. The stability of bacteriocin was tested in the presence of ethanol, methanol, isopropanol, acetone, ethyl acetate, sodium dodecyl sulphate (SDS), Tween 80, urea and Triton X-100 at a final concentration of 1% (v/v or w/v as appropriate), and incubated at 37 °C for 2 h. The bacteriocin LB44 was treated with proteinase K, trypsin and papain (1 mg/ml), and incubated at 37 °C for 2 h [11]. After incubation, the enzyme activity was terminated by heating at 100 °C for 5 min. The untreated bacteriocin was used as control for each treatment. The percent residual activity after treatments was determined in terms of AU/ml and compared with untreated control.

2.5. Effect on cell viability and potassium ion efflux

M. luteus (~10^6) was grown in NB medium containing bacteriocin LB44 (8 and 16 AU/ml) and incubated at 37 °C, 200 rpm in an incubator shaker. The untreated cells were grown under similar conditions and used as control. The samples were collected at regular interval of 2 h up to 12 h. The appropriate dilutions were spread plated on nutrient agar medium and incubated at 37 °C for 24 h. Cfu/ml of treated and untreated sets was compared to assess the loss of cell viability.

Potassium ion efflux of bacteriocin-treated cells of *M. luteus* was measured as described by Kumar et al. [13]. Briefly, *M. luteus* cells (~10^6) were washed with tris acetate buffer (10 mM, pH 7.0) containing 100 mM NaCl. After washing, cells were resuspended in same buffer containing bacteriocin LB44 (16 AU/ml). The potassium ion release was measured at every min up to 5 min using potassium-selective electrode calibrated with KCl solutions (20 and 40 ppm) fitted with a digital flame photometer (ESICO International, India). Nisin was used as positive control and bacteriocin diluent (Buffer I) as negative control.

2.6. Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM)

Effect of bacteriocin LB44 on cell membrane of indicator strain was studied using FTIR spectroscopy as described by Bizani et al.

Please cite this article in press as: R. Kaur, S.K. Tiwari, Membrane-acting bacteriocin purified from a soil isolate *Pediococcus pentosaceus* LB44 shows broad host-range, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.062