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Inhibiting bacterial colonization on catheters: Antibacterial and antibiofilm activities of bacteriocins from *Lactobacillus plantarum* SJ33

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ABSTRACT

Background: Catheter-associated urinary tract infections are one of the most common types of hospital-acquired infections that start with bacterial adhesion and lead to biofilm formation. The antagonistic activity of lactic acid bacteria against pathogenic organisms makes them important for medical applications.

Objective: This study evaluated the precise method for purification of bacteriocin from *Lactobacillus plantarum* subsp. *argentoratensis* SJ33, and its characterization and effectiveness for biofilm inhibition on urinary catheters coated with bacteriocin.

Methods: Purification of bacteriocin was carried out using various methods such as cell adsorption-desorption, gel permeation chromatography, and hydrophobic interaction chromatography. Bacteriocin preparation was analysed using reverse-phase high-performance liquid chromatography (HPLC) and further characterised by Tricine SDS-PAGE and Q-TOF ESI MS. Antibacterial activity of bacteriocin was assessed against 16 different Gram-positive and Gram-negative bacterial strains, and their effect on morphology was observed under scanning electron microscopy (SEM). Biofilm adherence and inhibition were evaluated by crystal violet assay, fluorescence microscopy and SEM.

Results and conclusions: Bacteriocin preparation exhibited broad-spectrum activity against both Gram-positive and Gram-negative bacteria, and SEM analysis revealed membrane pore formation. On treating with various enzymes, bacteriocin was found to be sensitive to proteases, which confirmed its proteinaceous nature. Bacteriocin showed its applicability at acidic pH in the urinary tract. Antibiofilm activity of bacteriocin established its significance in catheter-associated biofilm inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Molecular weight of bacteriocins, namely Bac F1 and Bac F2 as resolved by RP-HPLC, was estimated to be 4039 Da and 1609 Da, respectively.

1. Introduction

Catheter-associated urinary tract infections (CAUTIs) are some of the most common hospital-acquired infections. As a urinary catheter is the most commonly inserted medical device in acute-care patients, it has a higher possibility of bacterial adhesion during insertion. The potential effect of CAUTIs can be ascertained by the fact that these account for around 25% of infections in intensive care units and are responsible for around 50% of hospital infections [1]. One of the approaches to preventing CAUTIs is coating an antimicrobial compound on the surface of catheter to reduce bacterial adhesion; this is a promising method to curb CAUTIs. Due to increasing resistance of pathogenic bacteria against antibiotics, antimicrobial peptides like bacteriocins are considered safer and potent candidates [2].

Lactic acid bacteria (LAB) are known for producing bacteriocins, peptides, organic acid, and certain secondary metabolites. Bacteriocins from LAB are recognised for their ability to prevent microbial contamination and infections [3]. Several small peptide bacteriocins that are isolated and purified from different *Lactobacillus* strains have emerged as an alternative to antibiotics, due to their broad-spectrum antimicrobial activity in very low concentrations [2]. Bacteriocins from LAB are classified based on their structural, physiochemical, molecular characteristics and antibacterial activity [4] as: Class I bacteriocins – lantibiotics (e.g. Nisin), small (<5 kDa), membrane-active peptides; Class II bacteriocins – small, heat-stable, non-lanthionine-containing peptides; and Class III bacteriocins – large, heat-labile bacteriocins. Class II bacteriocins are divided into subclasses: IIa (pediocin-like bacteriocins, antilisterial activity); IIb (two peptide bacteriocins, e.g. lactococcin G); IIc

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(cyclic bacteriocins); and IId (single peptide, non-pediocin-like, linear bacteriocins). Class II bacteriocins act either by cell wall hydrolysis or dissipating membrane potential of the target organism [5].

Biofilm-associated infections on implantable medical devices caused by pathogenic strains, which have negative impacts on public health and medicine, are a major concern [6]. Alternatives are an immediate requisite to combat drugresistant pathogens [7]. Bacteriocins and antimicrobial peptides can be used as antibiofilm agents for combating infections as well as battling drug resistance.

This study reports purification, characterization and evaluation of antibacterial and antibiofilm activity of bacteriocin produced by *Lactobacillus plantarum* subsp. *argentoratensis* SJ33. A cell adsorption-desorption technique was used to purify bacteriocin, with minor modifications, and was further refined by gel permeation chromatography, hydrophobic interaction chromatography and reverse phase high-performance liquid chromatography (HPLC) to purify bacteriocin. The stability of bacteriocin was tested with different parameters (pH, temperature, chemicals and enzymes) and the antibacterial activity of bacteriocin was also assessed against various Gram-positive and Gram-negative bacteria. Ability of bacteriocin to prevent CAUTIs was demonstrated through impregnation coating of bacteriocin on urinary catheters and was assessed for antibacterial and antibiofilm activity.

2. Materials and methods

2.1. Bacteria strains, cell lines and growth conditions

Twelve pathogenic strains and four LAB strains listed in Table 1 were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. All LAB strains, including producer strain *Lactobacillus plantarum* subsp. *argentoratensis* SJ33, were grown in MRS (De Mann Rogosa Sharpe) medium. The pathogenic bacterial strains were grown in Tryptic Soy broth (TSB) and stored at −20 °C with 30% glycerol. The IEC-6 and HEK-293 cell lines were obtained from NCCS, Pune, India and grown in 90% Dulbecco's Modified Eagle Medium containing HEPES buffer and 10% FBS with 0.1% penicillin and streptomycin.

2.2. Bacteriocin purification

Lactobacillus plantarum subsp. *argentoratensis* SJ33 was grown in a modified production medium for 24 h at 37 °C [8] and the culture broth was processed by cell adsorption-desorption method. The SJ33 strain was grown in 2 L of optimised media in static conditions for 24 h at 37 °C. After incubation, pH of the culture was adjusted to 6.5 and kept at 70 °C for 15 min to inactivate proteases. The broth culture was kept at 4 °C for 4 h under agitation, then the cells were harvested at 10 000 × *g* for 15 min at 4 °C (Kubota, High speed Centrifuge, Japan). The cells were washed with 5 mM phosphate buffer (pH 6.5). Thereafter, the cells were resuspended in 100 mL 0.1 N NaCl, adjusted to pH 2.5 and kept for stirring at 4 °C for 2 h. The cells were then centrifuged at 10 000 × *g* for 15 min at 4 °C and the supernatant was recovered. The obtained supernatant was further desalted and purified by gel permeation chromatography (Sephadex G10 and G25 column) and the pooled active fraction from the column was passed through hydrophobic interaction chromatography, Sep-Pak C18 cartridge (Waters, Bangalore) equilibrated with water containing 0.1% trifluoroacetic acid and eluted with 20%, 30% and 50% of isopropanol to obtain various fractions with different polarity. The active fraction obtained from the C18 cartridge was further analysed in RP-HPLC (Shimadzu, Japan) for purity by injecting 100 μL of the bacteriocin sample. The reverse phase column (Waters, USA, C18) was pre-equilibrated with water (containing 0.1% trifluoroacetic acid). Bacteriocin fractions were eluted in a gradient of 0–30 min with solvent A (water containing 0.1% trifluoroacetic acid) and with solvent B (acetonitrile) at a flow rate of 0.5 mL/min and detected at 280 nm [9]. The quantity of protein in samples at every step of purification was estimated by the Bradford method [10].

2.2.1. Molecular mass determination

The molecular mass of bacteriocin preparation was determined using discontinuous Tricine SDS-PAGE (16% T and 6% C) [11]. An ultralow protein molecular weight marker of range 1.06–26.6 kDa (Sigma-Aldrich, India) was used as a standard and electrophoresed with bacteriocin purified from SJ33. One half of the gel was stained with Coomassie Brilliant Blue G-250 (Sigma-Aldrich, India) while the other half was fixed and overlaid on an agar plate with indicator strain [9]. The molecular weight of bacteriocin was confirmed by adding 1% formic acid and analysed on Q-TOF (Q-TOF SYNAPT G2) ESI mass spectrometer by direct infusion. Mass spectrometer calibrations were made by deconvoluting the raw data, which were processed by using Mass Lynx 4.1 WATERS software.

2.2.2. Bacteriocin assays and antibacterial activity

The activity of bacteriocin fractions obtained at each step was determined by two-fold serial dilution through agar well diffusion assay [12]. An overnight bacterial culture was grown and seeded into plates by the pour plate method; 10 μL of bacteriocin preparation (1600 AU/mL) was placed in 6 mm diameter wells and the plates were incubated for 24 h at 37 °C. The bacteriocin activity was expressed in arbitrary units (AU/mL), which was represented as being reciprocal of the highest dilution showing a distinct zone of inhibition. Bacteriocin (1600 AU/mL) was exposed to different temperatures (25–100 °C) for 1 h and 121 °C for 15 min. In another experiment, bacteriocin was treated with the proteolytic enzymes proteinase K, trypsin, chymotrypsin, carboxypeptidase, and amylase and lipase enzymes at 1 mg/mL concentration. Bacteriocin was also treated with different chemicals for 2 h at 37 °C to check for sensitivity. The bacteriocin that was obtained was tested for stability by adjusting the pH over a range of 2.0–10.0 and incubated for 2 h [13]. The spectrum of bacteriocin activity was tested against various Gram-positive and Gram-negative pathogens and some LAB strains given in Table 1 using the pour plate method.

2.3. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of bacteriocin preparation was evaluated against *Staphylococcus aureus* using

Table 1
Purification and activity of bacteriocin preparation produced by *Lactobacillus plantarum* subsp. *argentoratensis* SJ33.

Purification fraction	Volume (mL)	Total protein (mg)	Total activity (AU)	Specific activity (AU/mg)	Purification fold	Recovery (%)
Cell adsorption-desorption	10	45.4	8000	176.21	1	100
Gel filtration chromatography	2.5	1.54	4000	1038.96	5.9	50
Sep pak	0.5	0.5	1600	3200.00	18.16	20

Total protein refers to protein concentration (mg/mL) multiplied by the volume (mL).
Specific activity is arbitrary units (AU) divided by the total protein concenitration.

broth dilution assay by adding different concentrations of bacteriocin to microtiter plates (100 μL per well) and Nisin was taken as standard for comparison. The indicator strain added to each well was adjusted to 0.01 OD_{600nm} (i.e. 10^5CFU/mL) and incubated for 24 h at 37 °C. The MIC value was considered to be the lowest concentration at which bacteriocin completely inhibited the bacterial growth after 24 h. Bacteriocin added to TSB media without bacterial strain was taken as a positive control whereas bacterial culture without bacteriocin was considered as a negative control; both controls were maintained along with test samples.

2.4. Scanning electron microscopy

Staphylococcus aureus cells were viewed for morphological changes after treatment and incubation with bacteriocin preparation at MIC (4 mg/mL) for 4 h. The cells were washed with PBS and fixed with 2.5% glutaraldehyde. After fixation, cells were treated with 1% osmium tetroxide and finally dehydrated through a gradient series of ethanol (25–100%). For morphological analysis, the dehydrated cells were coated with gold, and examined under HR SEM (FEI Quanta FEG 200, USA).

2.5. Antibiofilm activity of bacteriocin

The bacteriocin effect on biofilm formation by *P. aeruginosa* and *S. aureus* was determined by crystal violet assay. Crude (CAD) sample and bacteriocin preparation were added at sub-MIC (1 mg/mL) along with overnight cultures of indicator strains (0.01 $O.D_{600nm}$) [14]. The microtiter plate was incubated for 24 h at 37 °C and absorbance was measured at 590 nm. The indicator strains without bacteriocin were considered as untreated. For microscopy, the coverslips treated with and without bacteriocin (1 mg/mL) were placed in 12-well plates containing cultures of *P. aeruginosa* and *S. aureus* (1:100 dilution) and incubated at 37 °C for 24 h, which were viewed under fluorescence microscope (Olympus, Japan) after staining with 0.01% acridine orange (w/v). Morphological changes on coverslips after biofilm formation and inhibition were analysed under HR SEM (FEI Quanta FEG 200, USA).

2.6. Inhibitory activity of bacteriocin on catheters

Urinary catheters (sterilised silicone Foley balloon catheter) were cut into segments and coated with bacteriocin preparation at MIC (4 mg/mL) via the impregnation process [15]. The coated and uncoated catheters were placed on petri plates swabbed with overnight culture (dilution 1:100) of indicator strains, and plates were incubated for 24 h at 37 °C. The agar plates were then observed for inhibition zone around the coated catheters [16]. Moreover, the uncoated catheter and bacteriocin coated catheter at sub-MIC (1 mg/mL) were also analysed for biofilm formation and inhibition by crystal violet staining. The catheter segments were fixed and stained for final viewing under a phase contrast microscope (Olympus, Japan).

2.7. Effect of bacteriocin on cell viability and morphology

The toxic effect of bacteriocin on viability of IEC-6 and HEK-293 cell lines was analysed using MTT assay [17]. Serial two-fold dilutions of bacteriocin (range 8–0.25 mg/mL) were prepared with culture media, which was added to the cells across the microtiter plate and incubated with 5% CO_2 for 24 h at 37 °C. The plate was supplemented with MTT (0.25 mg/mL) and incubated for 4 h; 200 μL DMSO was finally added and the absorbance was recorded at 570 nm. The morphology of the IEC-6 and HEK-293 cells was first observed by inverted microscope before the experiment and then the cells at 10^4 cells/mL density were incubated with and without different concentrations of bacteriocin. After incubation, the cells were stained with live/dead staining (AO/EB) [18] and viewed under a fluorescence microscope to determine the changes induced by bacteriocin.

3. Results

The bacteriocin-producing strain *Lactobacillus plantarum* subsp. *argentoratensis* SJ33 was characterized by 16S rRNA and multiplex PCR. The gene sequence was deposited in Genebank, NCBI, having accession no. JN573620 [19].

3.1. Purification and characterization of bacteriocin

Bacteriocin preparation was purified from SJ33 using a three-step purification method and the activity at each step was assessed. Bacteriocin activity (AU/mL) was represented as the inverse of the highest active dilution (Table 2). The crude sample obtained through CAD technique showed activity of 800 AU/mL (Table 2) and was desalted in a Sephadex G10 column and then passed through a Sephadex G25 column, in which the active peak showed bacteriocin activity of 1600 AU/mL (Table 2). The active peak obtained from the column was further purified by Sep-Pak cartridges and the bacteriocin obtained in 20% isopropanol eluted fraction exhibited 3200 AU/mL activity. The purified bacteriocin preparation was then analysed in reverse phase HPLC, where two peaks (BacF1 and BacF2) were resolved at retention times of 3.406 min and 4.897 min (Fig. 1B) and were present in a relative proportion of 2:3 as revealed by the corresponding peak areas. Tricine-SDS PAGE also showed a diffuse band between molecular masses 1.06 kDa and 6.5 kDa (Fig. 1(A) a). The molecular masses of bacteriocins BacF1 and BacF2 were confirmed to be 4039 Da and 1609 Da, respectively, by Q-TOF ESI mass spectrometry (Fig. 1C a and b), which corresponded to the two peaks resolved in reverse phase HPLC (Fig. 1B). The in-situ gel assay evidently revealed the antibacterial activity of bacteriocins by indicating zones of inhibition parallel to the gel bands (Fig. 1 (A) b).

3.1.1. Bacteriocin assays and antibacterial activity

Bacteriocin preparation from SJ33 showed wide-spectrum antibacterial activity against various Gram-positive and Gram-negative bacteria. Bacteriocin was found to be effective against different pathogens such as *S. aureus, Aeromonas hydrophila, Clostridium sporogenes, Clostridium perfringens, Escherichia coli* and human pathogens *Klebsiella pneumoniae and P. aeruginosa* (Table 1).

Table 2
Antibacterial spectrum of bacteriocin preparations produced by *Lactobacillus plantarum* subsp. *argentoratensis* SJ33 against bacterial strains.

Indicator strains	Zone of inhibition (mm)
Listeria monocytogenes MTCC 657	22 ± 2
Staphylococcus aureus MTCC 96	17 ± 1
Aeromonas hydrophila MTCC 1739	19 ± 2
Escherichia coli MTCC 728	18 ± 1
Clostridium perfringens MTCC 450	19 ± 1
Clostridium sporogenes MTCC 2684	20 ± 2
Bacillus subtilis MTCC 619	17 ± 1
Bacillus cereus MTCC 1272	18 ± 2
Vibrio parahaemolyticus MTCC 451	16 ± 1
Proteus vulgaris MTCC 426	16 ± 1
Klebsiella pneumoniae MTCC 3384	20 ± 2
Pseudomonas aeruginosa MTCC 3541	19 ± 1
*Lactobacillus fermentum*MTCC 1745	Nil
Lactobacillus rhamnosus MTCC 1408	Nil
Lactococcus lactis lactis MTCC 440	13 ± 1
Leuconostoc mesentroides MTCC 107	12 ± 1

Inhibition zone in mm, inclusive of well diameter 6 mm values are means ± SD of two independent experiments performed in triplicate.

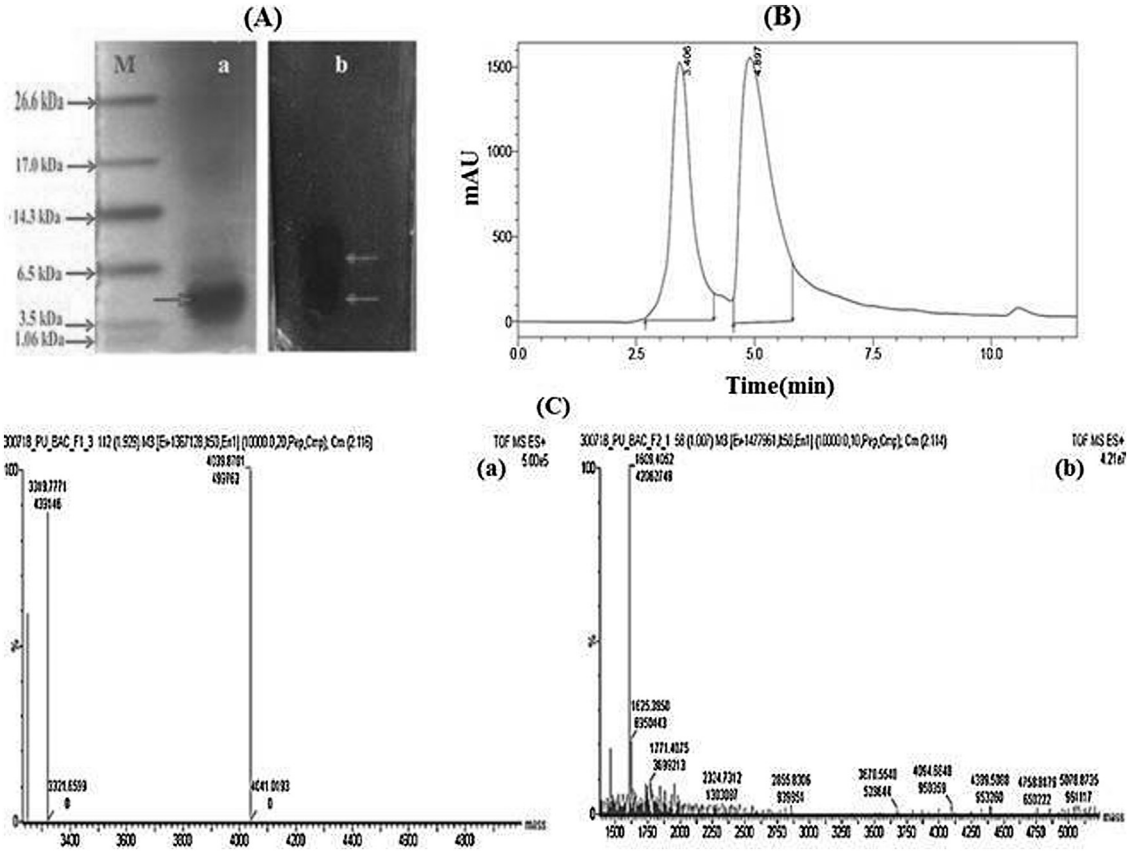

Fig. 1. (A) Tricine-SDS PAGE of bacteriocin samples (M is the ultra-low range molecular weight protein marker). a) bacteriocin preparation from SJ33, where arrows indicate bacteriocin and its corresponding activity; b) gel containing bacteriocin overlaid with indicator strain.
(B) Reverse phase HPLC (C18 column) profile of bacteriocin, where absorbance was monitored at 280 nm.
(C) Electron spray ionisation mass spectrum (Q-TOF ESI MS) analysis of both bacteriocins: a) Bac F1 and b) Bac F2.

However, bacteriocin was ineffective against some probiotic LAB strains. Bacteriocin was observed to be acid tolerant and thermostable, as the activity was not lost even after exposure for 15 min at 121 °C (Supplementary data Table 1). Bacteriocin showed no activity when treated with proteases (Supplementary data Fig. 1) confirming its proteinaceous nature, whereas antibacterial activity was retained on treatment with lipase and amylase.

3.2. Determination of minimum inhibitory concentration

The MIC of bacteriocin preparation was determined against *S. aureus*, which showed nearly 90% inhibition of *S. aureus* at 4 mg/mL and around 94% at 5 mg/mL bacteriocin treatment, while around 95% reduction was found at 4 mg/mL of Nisin (Fig. 2). These results suggest that bacteriocins are effective against *S. aureus* and no significant difference between inhibitory concentration of bacteriocin and Nisin was observed.

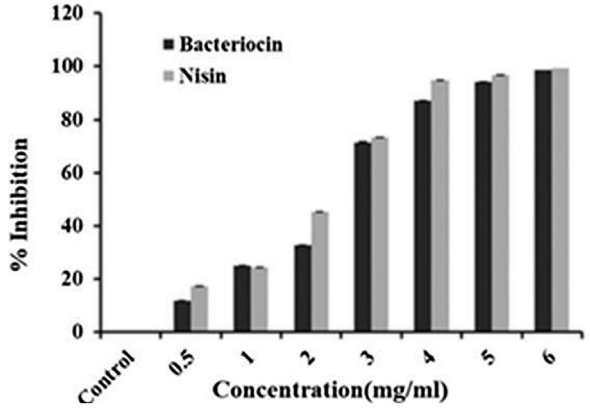

Fig. 2. Determination of MIC of bacteriocins against *S. aureus*. Nisin was used as a standard. Results of two independent experiments in triplicate are presented as mean and standard deviation.

3.3. Scanning electron microscopy

The SEM results indicated altered cell morphology in bacteriocin-treated *S. aureus* compared with untreated cells. The untreated cells were intact, turgid and separated from one another (Fig. 3A), whereas cells treated with bacteriocin preparation at MIC were deformed and had prominent pores on the cell membrane (Fig. 3B). Scanning electron micrographs showed pore formations and leakage of cellular contents, which confirmed the bactericidal nature of bacteriocin.

3.4. Antibiofilm activity of bacteriocin

The untreated biofilms of *P. aeruginosa* and *S. aureus* showed OD_{600nm} 2.0 and 1.5, respectively. After bacteriocin treatment, 56% of inhibition was found in biofilm formed by *P. aeruginosa* and 62% reduction in biofilm formation by *S. aureus*. However, the crude sample reduced 44% of biofilm by *P. aeruginosa* and 15% of biofilm formation by *S. aureus* (Supplementary data Fig. 3). The bacteriocin significantly inhibited biofilm formation, which was viewed under fluorescence microscope (Fig. 4) and these results were consistent

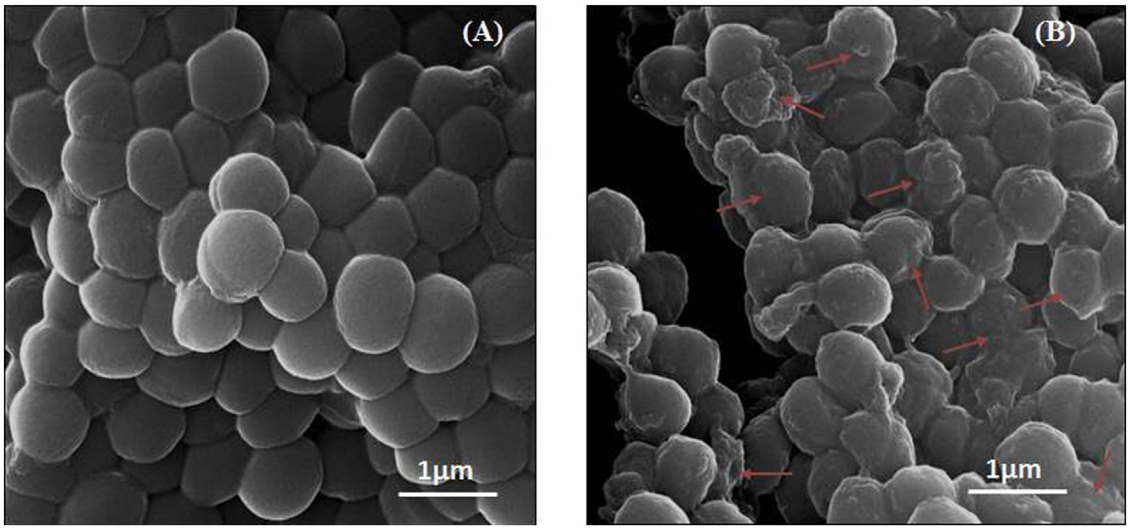

Fig. 3. Scanning electron micrograph images of *Staphylococcus aureus* cells show (B) membrane pore formation after bacteriocin treatment (indicated by arrows) compared with (A) untreated cells.

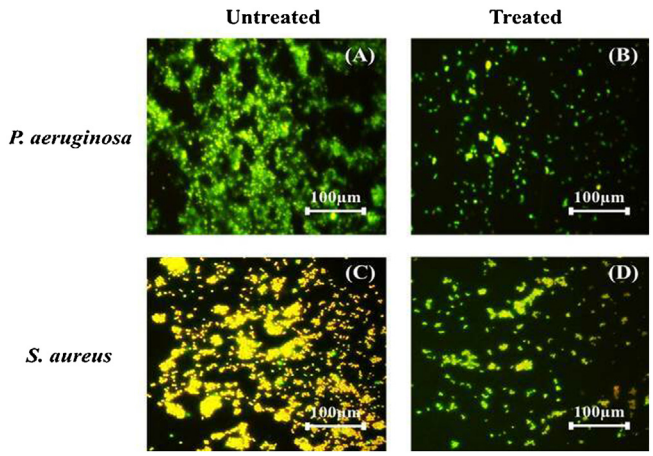

Fig. 4. Biofilms of 24 h *Pseudomonas aeruginosa* and *Staphylococcus aureus* grown on coverslips and viewed under fluorescence microscope. (A) and (C) untreated biofilms; (B) and (D) treated with sub-MIC (1 mg/mL) of bacteriocins for 24 h.

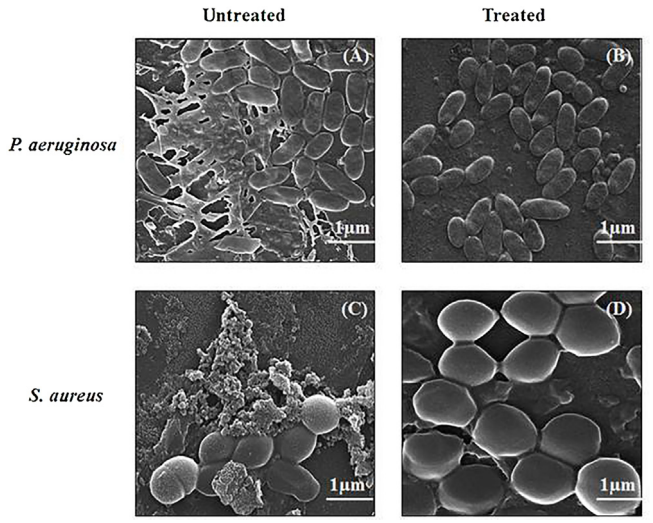

Fig. 5. Scanning electron micrographs shows biofilms formation by (A) *Pseudomonas aeruginosa*, (C) *Staphylococcus aureus* (untreated), and (B) and (D) biofilm treated with bacteriocin sub-MIC (1 mg/mL).

with the findings of crystal violet assay (Supplementary data Fig. 2). The scanning electron micrographs of biofilm formation and inhibition by bacteriocin on coverslips are shown in Fig. 5. For untreated samples of both strains, the biofilms were uniform and dense, and an extracellular layer was formed (Fig. 5A and C), while the biofilms treated with bacteriocin preparation (1 mg/mL) were found to be individual and discrete colonies (Fig. 5B and D).

3.5. Inhibitory activity of bacteriocin on catheters

Zone of inhibition was observed around the catheters coated with bacteriocin preparation (MIC at 4 mg/mL) against both *P. aeruginosa* and *S. aureus* as compared with uncoated catheters (Fig. 6B and C). The inhibitory zone was clearer against *S. aureus* (Fig. 6C) in comparison with *P. aeruginosa* (Fig. 6B). Notable differences in bacterial adherence and biofilm inhibition were observed between coated and uncoated catheters against biofilms of both pathogens (Fig. 6B and C).

3.6. Effect of bacteriocin on cell viability and morphology

Cytotoxicity of bacteriocin assessed by MTT assay on IEC-6 and HEK-293 cell lines showed toxic effect only at higher concentrations (8 mg/mL) (Supplementary data Fig. 3). The viability increased significantly with decreases in concentration: >90% cell viability was found up to 0.25 mg/mL and nearly 50% and 58% of cell viability at 8 mg/mL. Cells viewed with live/dead staining revealed no significant change in IEC-6 and HEK-293 cells after bacteriocin treatment when compared with untreated cells (Supplementary data Fig. 3).

4. Discussion

The present study mainly focused on purification, characterization, and evaluation of antibiofilm activity of low molecular weight bacteriocin produced by *Lactobacillus plantarum* subsp. *argentoratensis* SJ33. Bacteriocin production was found to be better in optimised media than in MRS media, as previously reported [8]. The bacteriocin from SJ33 was purified in three steps, which included cell adsorption-desorption, gel permeation chromatography, and hydrophobic interaction chromatography. The specific activity increased from 176.21 to 3200 (AU/mg) as purification was achieved, resulting in 18-fold purification and 20% final recovery of bacteriocin (Table 2). Purification methods for bacteriocins are

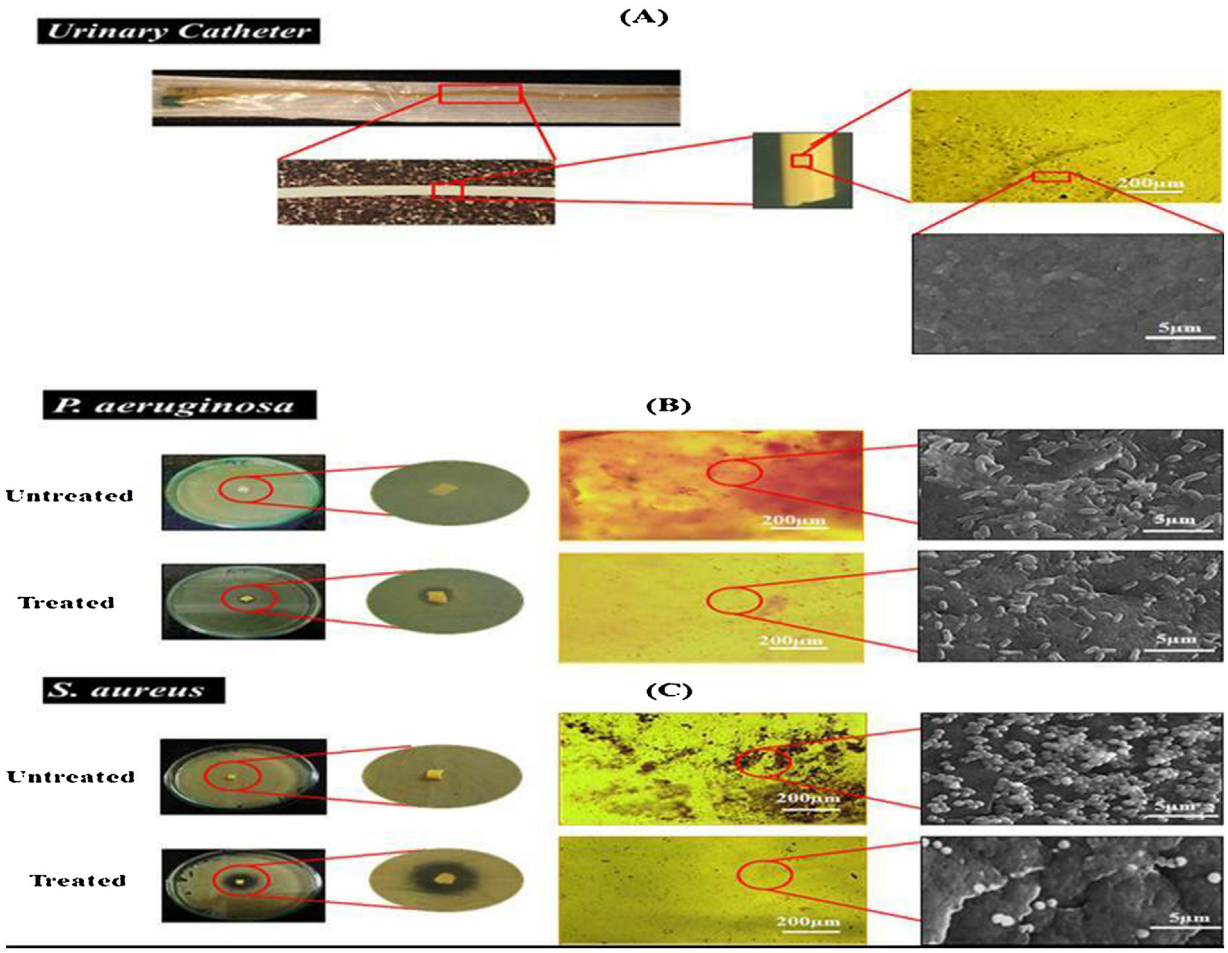

Fig. 6. (A) Sterilised silicone Foley urinary catheters and segments of catheters and catheter surface. Antibacterial activity at MIC (4 mg/mL) and antibiofilm activity at sub-MIC (1 mg/mL) of bacteriocins on catheters against: (B) *Pseudomonas aeruginosa* and (C) *Staphylococcus aureus* was observed by phase contrast and scanning electron microscopy. Biofilm formation and inhibition was observed by phase contrast and scanning electron microscopy.

generally cumbersome processes, usually involving precipitation followed by different combinations of chromatographic techniques that usually vary from three or more steps. The nature of class II bacteriocins makes purification tedious, mainly for those produced by *Lactobacillus* spp. [20,21]. Tricine SDS-PAGE analysis showed the presence of two bacteriocins of molecular mass <5.0 kDa but could not predict the accurate difference between the masses. The purified bacteriocin preparation was further resolved to BacF1 and BacF2 by RP-HPLC, and found to be individually active and at equimolar concentrations; BacF1 was found to be more potent than BacF2, as observed by agar well diffusion assay (Supplementary data Fig. 4).

These results were in agreement with an earlier study on bacteriocins produced from LR/14 [22] and bacteriocins from *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *Carnobacterium piscicola* and *Enterococcus faecium* [23], which reported production of more than one bacteriocin. *Lactobacillus sakei* produced three bacteriocins, namely: 5T, 5X and 5P [24]. *Leuconostoc mesenteroides* produced three bacteriocins: leucocin A, leucocin B and leucocin C of molecular weight 3933 Da, 3466 Da and 4598 Da, respectively [24,25]. *Carnobacterium piscicola* produced bacteriocins, namely piscicocin V1a and piscicocin V1b of molecular weights 4416 Da and 4526 Da, respectively. *Enterococcus faecium* L50 produced four bacteriocins: L50A, L50B, Q and P. Bacteriocins NKR-5-3A, NKR-5-3B, NKR-5-3C and NKR-5-3D were produced by *Enterococcus faecium* NKR-5-3 [26].

The purified bacteriocin in this study exhibited a broad range of antibacterial activity against Gram-positive and Gram-negative bacteria, including *S. aureus*, *Escherichia coli*, *Bacillus cereus*, *Aeromonas hydrophila* and *P. aeruginosa*. Some bacteriocins from *Lactobacillus* spp. are reported to show better antibacterial activity against Gram-negative pathogens [27], while other bacteriocins could not inhibit Gram-negative bacteria [28,29], which suggests that different bacteriocins are produced from different LAB strains. The bacteriocin preparation was observed to be acid tolerant and thermostable, as the activity was not lost even after exposure at 121 °C for 15 min, which makes it a good candidate for pharmaceutical applications. The class II bacteriocins are normally heat-stable small peptides and similar reports were found in cases of plantaricin LP31, sakacin M, sakacin P, acidocin D20079, pediocin L50, pediocin NV 5 and piscicocin CS526 [21,24]. Bacteriocin F1 from *Lactobacillus plantarum* was also stable at 121 °C for 10 min [13] but bacteriocin F4 lost activity after exposure for 30 min at 90 °C [22]. Complete loss of activity was observed when bacteriocin was treated with proteolytic enzymes like α-chymotrypsin and trypsin and other proteases, confirming its proteinaceous nature. Similar activity was observed in bacteriocins produced by *Pediococcus acidilactici* and other bacteriocins from various *Lactobacilli* strains, which were reported to produce proteinaceous antimicrobial compounds [9,30,31] and those remained unaffected by treatment with lipase and α-amylase [32,33]. Interestingly, reagents, detergents and other

chemicals had no significant effect on the antibacterial activity of bacteriocin.

The purified bacteriocin preparation showed membrane disruptions and pores on bacterial cell membranes, indicating squeezing out of the inner contents of the cells. Scanning electron micrographs confirmed the pore formations and bactericidal nature of the bacteriocin. The inhibitory effect of each bacteriocin from diverse LAB strains may differ against various pathogenic bacteria. Bacteriocins are either bactericidal or bacteriostatic in nature [34] (i.e. some bacteriocins are reported to be bacteriostatic against one pathogenic strain and bactericidal against the other) [22]. Several mechanisms of action for class II bacteriocins were proposed, while the general mechanism of bacteriocin action was pore formation leading to leakage of cellular materials [35,36].

Biofilms have become a major problem in healthcare activities and new antibiotics are urgently required to reduce the growing hazard from biofilm-forming pathogens that are resistant to commercial antibiotics [37]. The present study revealed the antibiofilm potential of purified bacteriocin on inhibition of biofilm formation by *P. aeruginosa* and *S. aureus*, which have been classified as strong biofilm formers based on crystal violet assay. The effect of bacteriocin on biofilms at sub-inhibitory concentrations was examined by a co-inoculation experiment, which resulted in significant reductions in biofilm formation, whereas inhibition of biofilms treated with crude samples was found to be poor. The study showed an increase in the percentage of inhibition as bacteriocin purity increased. Moreover, bacteriocin even at lower concentrations inhibited biofilm formation. Earlier works on mechanisms of antimicrobial peptides and polysaccharides were unable to predict the accurate mechanisms by which they break-down biofilms [38].

The current study on effect of bacteriocin against CAUTIs showed antibacterial activity of bacteriocin coated catheters against *P. aeruginosa* and *S. aureus* using the agar plate method, while the antibiofilm activity of bacteriocin against both the pathogens was observed under phase contrast microscope using crystal violet staining and through scanning electron microscope. It was found that a bacteriocin coating does not affect the quality of catheters, so their handling will not be affected during clinical use. The most effective method for direct prevention of CAUTI was to coat the surface and luminal parts of catheters with antimicrobial compounds, hence providing dual protection against infections [38] that tend to reduce bacterial load. However, bacteriocins from LAB have previously reported strong bactericidal activity, effective biofilm inhibition and nontoxic nature.

The cytotoxic study of bacteriocin showed ~60% viability at MIC on IEC-6 and HEK-293 cell lines in comparison with untreated cells. No remarkable change was observed in the morphology of cells of treated samples at different concentrations as compared with untreated cells after 24 h. However, a decrease in cell number along with some deformed cells was noticed at higher concentrations of bacteriocin treated cells. A previous study on bacteriocins from *Bacillus* spp. showed 48% and 91% cell viability on HT29 and HEK293 cells, respectively, and the crude antimicrobial compound showed 6% cytotoxicity against Caco-2 cells [39]. Nisin, a common commercially available bacteriocin, was also reported to be mildly toxic on normal mammalian cells [40] but at higher concentrations. Bacteriocins have been reported for their variable effect on mammalian cell lines and these are due to different factors but the actual mechanism involved in cytotoxicity is not yet entirely understood.

5. Conclusion

Bacteriocin produced from *Lactobacillus plantarum* subsp. *argentoratensis* SJ33 showed broad-spectrum antibacterial activity against numerous Gram-positive and Gram-negative pathogens. Bacteriocin was heat tolerant and exhibited activity over acidic to neutral pH. This study has led to purification of two bacteriocins possessing significant assets, like thermostability, bactericidal action and interestingly reported antibiofilm activity against biofilm-forming pathogenic strains such as *P. aeruginosa* and *S. aureus*. Catheters coated with bacteriocin showed prevention of bacterial colonisation associated with UTIs, which was confirmed by scanning electron micrographs. This highlights the medical uses of bacteriocin and opens new opportunities in the health sector. Bacteriocin showed no significant cytotoxicity up to the inhibitory concentration against normal mammalian cell lines. Bacteriocin possesses most of the desirable properties, which make it an exceptional prospect for application as a natural therapy and can also be considered as future biomedicine. The above work has opened an opportunity for potential biotechnological and clinical applications of bacteriocins. Further complete characterization of the purified bacteriocins is required to determine and confirm the novelty of these bioactive antibacterial compounds.

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Competing interests

None.

Ethical approval

Not required

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.02.021.

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