Partial characterization of heat stable, antilisterial and cell lytic bacteriocin of *Pediococcus pentosaceus* CFR SIII isolated from a vegetable source

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ABSTRACT

Heat-stable, antilisterial and cell lytic bacteriocin producing *Pediococcus pentosaceus* CFR SIII isolated from vegetable source (cucumber) was partially characterized. The isolate was identified by microbiological methods and 16S rRNA gene sequences. The bacteriocin produced by this isolate, designated as PP SIII, was active against several Gram-positive and Gram-negative food borne pathogens and food spoilage lactic acid bacteria. The apparent molecular mass of the partially purified bacteriocin was found to be ~5 kDa by Tricine SDS-PAGE. It was stable at pH 3-5 and at 121° C for 15 min and inactivated by various proteases. Mode of action of the bacteriocin through FTIR analysis and glycolytic activity assay revealed cell lytic activity against the indicator *P. acidilactici* B1153 by complete cell lysis, depletion of intracellular solute and disruption of pH gradient. The study envisages the potentiality of the isolate in vegetable preservation or as an adjunct culture in various cheese varieties to avoid chemical preservatives.

Keywords: bacteriocin · cell lytic · characterization · molecular weight · *Pediococcus*

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INTRODUCTION

In recent years, the consumption of foods formulated with chemical preservatives has increased consumers concern due to health effects and created a demand for more natural and minimally processed foods. As a result, there has been a great interest in naturally produced antibacterial agents for their application in food preservation (Cleveland et al. 2001). Lactic acid bacteria (LAB) are industrially important group of microorganisms, with GRAS status and are associated with meat, dairy and vegetable fermentations. Bacteriocins, short chain peptides having antimicrobial activity, can act as natural preservatives. Bacteriocins reduce the risk of food born diseases and outbreaks and increase safety of the food. Extensive reviews are available on the bacteriocins of LAB including that of *Pediococcus* and their application in the control of spoilage and pathogenic bacteria (Cintas et al. 2001; Jeevaratnam et al. 2005; Gálvez et al. 2008).

Pediococcus, a homofermentative LAB, is being used as an acid producing starter culture in sausage, sauerkraut, cucumber and green bean fermentations, soya milk fermentations and silage (Simpson and Taguchi 1995) and as a probiotic culture in the feed formulations for monogastric animals (Chaucheyras-Durand and Durand 2010). Isolation of pediocin or pentocin type of bacteriocin producing *P. pentosaceus* has been reported from different sources such as wine, sausage, refrigerated pork, grape juice, cucumbers, beans and human faeces (Strasser de Saad and Manca de Nadra 1993;Wu et al 2004; Halami et al. 2005; Shin et al. 2008;Uymaz et al., 2009; Venkateshwari et al. 2010). Partial characterization of pediocins isolated from several species of *Pedicoccus*, such as *P. acidilactici* NCIM 2292, *P. pentosaceous* NCIM 2296 and *P. cerevisiae* NCIM 2171 was reported earlier (Jamuna and Jeevaratnam, 2004). *Pediococcus parvulus*, which had an inhibitory effect on *Enterobacteriaceae* was isolated from Xuanwei ham, a Chinese fermented meat product (Li et al. 2008). Production of bacteriocin pediocin PA1 by vegetable associated *P. parvulus* was noticed (Bennik et al 1997). Bacteriocins of *Pediococcus* are small, heat stable and non-lanthionine containing peptides belonging to the class II that was proposed by Klaenhammer (1988). Use of pediocin along with other process technologies has been proposed to reduce the process severity (Balasubramaniam and Farkas 2008). Several aspects of bacteriocins produced by pediococci have been reviewed (Papagianni and Anastasiadou 2009). Earlier, purification and characterization of bacteriocin from *Pediococcus pentosaceus* ACCEL was reported (Wu et al. 2004). Very recently ccharacterization of the heat stable bacteriocin produced by vancomycin-sensitive *Pediococcus pentosaceus* CFR B19 isolated from beans

was reported (Venkateshwari et al. 2010). Numerous studies on the mode of action have been performed on peptide bacteriocins. Bacteriocins like pediocin D, nisin A and Z are membrane active, causing permeabilisation and eventually killing the target cells by interrupting cell wall synthesis through high affinity binding to lipid II molecule, a molecule that plays an essential role in the synthesis of the peptidoglycan layer (Hasper et al. 2006). According to Nilsen et al. (2003) bacteriocin zoocin- A from *Streptococcus zooepidermicus* 4881 causes hydrolysis of specific peptide bonds on the surface or interpeptide bridges in the peptidoglycan of susceptible bacteria such as *Pediococcus*, *Enterococcus*, *Lactococcus* and *Lactobacillus*. Cell lytic activity of pediocin AcH/PA-1 produced by *P. acidilactici* and *P. pentosaceus* was detected against cells of *Lactococcus lactis* subsp. *lactis*, *L. delbrueckii* subsp. *bulgaricus*, *Lactobacillus helveticus* and *Listeria monocytogenes* (Mora et al. 2003). However, Todorov and Dicks (2005) reported that pediocin ST18 produced by *P. pentosaceus* ST18 had bacteriostatic action towards *Listeria innocua*, with no cell lysis. Mode of action of several bacteriocins of LAB has been reviewed exhaustively (Montville and Chen, 1998; McAuliffe et al. 2001; Bauer and Dicks, 2005; Bauer et al. 2005) and the effective use of bacteriocins in food preservation requires the understanding of their mode of action and inhibitory action under different biochemical conditions naturally occurring in food (De Vuyst and Vandamme 1994; O'Sullivan et al. 2002).

The aim of the present study was to identify the *P. pentosaceus* CFR SIII isolated from vegetable source and to determine the mode of action of the partially characterized bacteriocin. This is the first report on production of complete cell lytic, heat stable, antilisterial bacteriocin by *P. pentosaceus.*

Materials and Methods

Fine chemicals and reagents

All chemicals were purchased from Sisco Research Laboratories, Mumbai, India. Antibiotics and all microbiological media used in this study were purchased from Hi-Media Laboratories, Mumbai (India). Proteolytic enzymes such as Proteinase-K, Papain, Trypsin and dithiothreitol were purchased from SIGMA (USA). Nisin was procured from ICN Biochemicals (USA). The organic solvents such as acetone, chloroform and Methanol were obtained from Qualigens, Mumbai, India.

Table-1

Bacterial cultures and growth conditions

Bacterial strains used in this study are listed in Table 1. *P. acidilactici* K7 (Halami et al. 2005), *Enterococcus faecium* MTCC 5153 (Halami 2010) and *P. pentosaceus* CFR SIII were previously isolated from vegetable source (cucumber) in our laboratory based on its antibacterial activity and deposited at the culture collection repository of Food Microbiology department, CFTRI, Mysore. All LAB cultures were grown in *Lactobacillus* deMan-Rogosa-Sharpe (MRS) broth and pathogenic bacteria were grown in brain heart infusion (BHI) broth at 37°C.

Bacterial strain identification and phylogeny

Bacteriocin producing *P. pentosaceus* CFR SIII was subjected to microbiological and biochemical assays for taxonomic identification (Garve 1986). The 16S rRNA gene amplification was carried out using the primers and PCR conditions described previously (Halami et al. 2005). The 16S rRNA gene was cloned into PGEMT vector and sequenced using M13 vector primer. The sequence generated was BLAST searched (Altschul et al. 1997). The phylogenetic analysis was carried out and dendrogram was constructed using MEGA version 3 software with Kimura 2 parameter model using 1000 bootstrap replicates (Kumar et al. 2004).

Antibiogram

To evaluate the antibiotic sensitivity of the bacteriocin producing isolate, the octodiscs (a ready to use 8-in-one antibiotic combination module, Hi-Media Laboratories Ltd., India) were placed on MRS agar seeded with the test cultures and incubated at 37°C for 24 h. The plates were observed for zone of inhibition and the cultures were classified as resistant or sensitive based on cut off antibiotic concentration as per the data provided by the manufacturer.

Antibacterial activity of the culture filtrate

Pediococcus pentosaceus CFR SIII was grown over night in MRS broth at 37°C and the cells were removed by centrifugation at 6500 *x g*. The pH of the cell free culture was adjusted to 7.0 using 1 N sodium hydroxide. The antibacterial activity of this neutralized filtrate against the indicator strain *L. monocytogenes* Scott A was determined by using agar well diffusion assay (Geis et al. 1983).

120 Bacteriocin production and partial purification

121 *Pediococcus pentosaceus* CFR SIII was grown in MRS broth for 16 h at 37°C and centrifuged at
122 6500 *x g* for 10 min. The supernatant was mixed with equal volume of chloroform using a magnetic
123 stirrer. The chloroform extract was separated by centrifugation and concentrated in a lyophilizer. The
124 chloroform extract was resuspended in sterile distilled water. The bacteriocin preparation was
125 designated as PP-SIII. Bacteriocins from *Pediococcus acidilactici* K7 and *Enterococcus faecium*
126 MTCC 5153 were purified from 16 h cultures grown in MRS broth as indicated above.

127

128 Characterization of bacteriocins

129 The antibacterial activity of the partially purified bacteriocin of *P. pentosaceus* CFR SIII was tested
130 using agar well diffusion assay by following the method of Geis et al. (1983) with *P. acidilactici*
131 B1153 as indicator, as well as other pathogens and lactic cultures as listed in Table 1. Effect of
132 proteolytic enzymes such as trypsin, proteinase-K, papain, lysozyme, peptidase and protease at a
133 concentration of 1mg/ml, pH (2-10), temperature (50 to 121°C for 15 min) and 10% β-mercaptoethanol
134 (β-ME) on antibacterial activity was tested as described previously (Halami et al. 2005). The
135 bacteriocin preparation having an activity of 100 AU ml^{-1} was taken for different treatments and the
136 residual bacteriocin activity after treatment was assayed against the indicator strain *L. monocytogenes*
137 Scott A.

138

139 Analysis of bacteriocins by Tricine SDS-PAGE

140 The bacteriocin preparation obtained from *P. pentosaceus* CFR SIII was redissolved in buffer and
141 separated by Tricine SDS-PAGE (16%) as described by Schagger and Von Jagow (1987). Samples
142 were run in duplicate along with the low molecular weight (Mw) marker (Sigma, USA). One half of the
143 gel was stained with silver staining and other half of the gel was washed extensively with sterile
144 distilled water. Bacteriocin bands were identified by overlaying the gel on BHI agar plate seeded with
145 *L. monocytogenes* Scott A.

146

147 Release of UV absorbing solutes

148 To study the putative mode of action of bacteriocin of *P. pentosaceus* CFR SIII, release of UV
149 absorbing material from the bacteriocin treated *L. monocytogenes* Scott A was studied by following a

method described earlier (Motta et al. 2008). For this the cell pellet of *L. monocytogenes* was treated with 2000 AU ml^{-1} of the partially purified bacteriocin of *P. pentosaceus* CFR SIII for 4 h. The treated cell suspension was filtered through 0.22 μ filter membrane (Millipore, USA). The filtrate was checked for absorbance at 260 nm and 280 nm using a UV-visible spectrophotometer (UV 400, Shimadzu, Japan). For comparison purpose, the cells *L. monocytogenes* Scott A were treated similarly with the pediocin preparation of same concentration from *P. acidilactici* K7 (Halami et al 2005) and enterocin from *E. faecium* MTCC 5153.

Scanning Electron Microscopy (SEM)

To study the morphology of the cultures and to determine the mode of action of the bacteriocin produced by *P. pentosaceus* CFR SIII the SEM analysis was carried (McDougall et al. 1994). To know the effect of bacteriocin on cell morphology, the cell pellet of *Pediococcus acidilactici* B1153 grown in MRS broth for 12 h at 37°C was suspended in the bacteriocin preparation (2000 AU ml^{-1}) and incubated for 1 h. The bacteriocin treated and untreated cells were processed for SEM. The cells were harvested by centrifugation at 6500 *x g* for 15 min and were fixed using 2.5% (v/v) aqueous glutaraldehyde for 2 h. These cells were dehydrated using a gradient of ethyl alcohol (10 -100%) and final wash was done with absolute ethyl alcohol. The dried cells were gold plated and subjected to scanning electron microscopy (LEO 435-VP, England, UK).

Effect of bacteriocins on glycolytic activity of *L. monocytogenes* Scott A

The effect of bacteriocin on glycolytic activity was studied by measuring the alteration in pH in bacteriocin treated *L. monocytogenes* Scott A suspended in glycolytic buffer with 0.5% glucose or maltose. The cell pellet of exponential phase culture of *L. monocytogenes* Scott A was washed with 0.5 mM phosphate buffer (pH 6.5) containing 70 mM potassium chloride and 1 mM magnesium sulphate. The cell pellet was equilibrated with the buffer and stored at $0^{o}C$ until use. The equilibrated cells were energized with fermentable substrate like glucose or maltose (0.5%) and treated with the bacteriocin PP-SIII having an activity of 2000 AU ml^{-1}. The change in pH was recorded from 0 to 30 min in cells treated with the bacteriocin PP-SIII. The pH change in cells treated with 2000 AU ml^{-1} each of bacteriocins of *P. acidilactici* K7, *E. faecium* MTCC 5153 and nisin at a concentration of 0.1 mg ml^{-1}, was observed. The cells suspended in glycolytic buffer without any bacteriocin served as a control.

FTIR spectroscopy of the bacteriocin

To study the mode of action of the bacteriocin PP-SIII on cell membrane, the bacteriocin treated *P. acidilactici* B 1153 was subjected to FTIR analysis. For this, the cells were pelleted and treated with 2000 AU ml^{-1} of bacteriocin preparation of *P. pentosaceus* CFR SIII. The treated and untreated cells of the test organism were washed thrice with distilled water. The washed cells were lyophilized to remove moisture and powdered. The cells were mixed with finely grounded potassium bromide and FTIR spectrum was recorded using FTIR spectrometer (Perklin Elmer, USA).

Results and discussion

Taxonomical identification of the isolate

Microbiological tests in combination with 16S rRNA gene sequencing clearly revealed the taxonomic identification of the bacterial isolate. The isolate was found to be Gram-positive, non-motile, catalase negative tetra-coccus. It could not hydrolyze starch, gelatin and citrate. It produced acid and gas from lactose, ribose and maltose, but not from xylose and mannose. It could grow at 45°C but not at 50°C. It did grow with 4% Sodium chloride, but not with 0.04% sodium azide and 3.5% potassium tellurite. Three major clusters of pediococci, enterococci and lactococci were obtained by the 16S rRNA phylogeny (data not shown). The phylogeny clearly identified the isolate at the species level. Based on microbiological, physiological tests and 16S rRNA phylogeny, the isolate *P. pentosaceus* CFR SIII was identified as *P. pentosaceus* and assigned with *P. pentosaceus* CFR SIII. The 16S rRNA gene sequence was submitted to GenBank with accession number FJ966190.

Antibacterial spectrum of *P. pentosaceus* CFR SIII

In the preliminary screening, the cell free filtrate of *P. pentosaceus* CFR SIII was found to inhibit the growth of the pathogenic *L. monocytogenes* Scott A with a 12 mm inhibition zone. In order to determine the antibacterial spectrum of the isolate, in subsequent studies, activity of the bacteriocin preparation was tested against a series of indicator bacteria. Antibacterial activity of *P. pentosaceus* CFR SIII is given in Table 1. The bacteriocin of *P. pentosaceus* CFR SIII exhibited antibacterial activity against wide range of pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella paratyphi*, *S. typhi*, *Yersinia* and *Leuconostoc mesenteroides*.

Antibiogram

In antibiogram test, the isolate was found to be sensitive to ampicillin, erythromycin, chloramphenicol, novobiocin, nitrofurantoin except gentamycin and nalidixic acid. Resistance against vancomycin was also observed with *P. pentosaceus* CFR SIII, as the pediococci are known to be intrinsically resistant to vancomycin (Swenson et al. 1990). However, recently a vancomycin-sensitive *Pediococcus pentosaceus* CFR B19 was isolated from beans (Venkateshwari et al. 2010). In recent years spread of antibiotic resistance is primary concern for food technologist and health care professional. Sensitivity of the isolate to common antibiotics and the intrinsic resistance of *P. pentosaceus* CFR SIII against the vancomycin alleviate the health concern regarding genetic transfer of antibiotic resistant genes and make the isolate safe for exploring as starter culture in food fermentation.

Characterization of the bacteriocin

The bacteriocin preparation from the isolate showed distinctive characteristics with respect to pH and temperature stability as well as degradation by proteolytic enzymes. The bacteriocin of *P. pentosaceus* CFR SIII lost activity only with trypsin and proteinase K treatment. Antimicrobial activity was completely lost upon the treatment with 10% β-ME, indicating the protenaceous nature of the bacteriocin. It was active in the acidic pH range of 3-5. It completely lost its activity at pH 2 and above 6.The bacteriocin from *P. pentosaceus* CFR SIII was found to be heat stable as it showed resistance after the treatment at 121°C for 15 min. Similarly, more than 80% activity of the bacteriocin of *P. pentosaceus* ACCEL was left after 15 min of heating at 121 °C (Wu et al. 2004) and the bacteriocin produced by *Pediococcus pentosaceus* CFR B19 showed resistance when subjected to similar treatment (Venkateshwari et al. 2010). Tricine SDS-PAGE (Fig 1) analysis revealed the zone-producing band corresponding to an apparent MW of ~5 kDa. Similarly, production of bacteriocin with a Mw of ~ 4.8 kDa by *Pediococcus pentosaceus* CFR B19 (Venkateshwari et al. 2010), pediocin PA-1 having a Mw of 4.6 kDa by *Pediococcus acidilactici* PAC 1.0 (Henderson et al. 1992) and pediocin PD-1 having a Mw of ~2.6 kDa was noticed (Bauer et al. 2005). However, production of bacteriocin having a higher Mw (17.5 kDa) by *P. pentosaceus* ACCEL was also reported (Wu et al. 2004). *Pediococcus* is widely associated with the fermentation of meat and vegetables. The two species *P. acidilactici* and *P. pentosaceus* are known to produce bacteriocins similar to lantibiotics active against *Listeria*, LAB and numerous pathogens prompting its use as starter culture in fermented meat products.

Fig 1

Mode of action of the bacteriocin PP-SIII

The SEM of *P. acidilactici* B1153 cells treated with the bacteriocin PP-SIII is shown in Fig. 2. As observed by SEM, the primary mode of action of PP-SIII was found to be cell lysis, which is being reported for the first time. However, the bacteriocins of *Pediococcus* are known to effect bactericidal activity through pore formation in the cytoplasmic membrane (Bhunia et al. 1991). To substantiate the mode of action, the effect of the bacteriocin on glycolysis and FTIR analysis of bacteriocin treated listeria were studied along with nisin as well as bacteriocins produced by *P. acidilactici* K7 and *E. faecium* MTCC 5153 as positive controls.

Fig. 2

a) Release of UV absorbing materials

Increase in UV absorbance at 280 nm (0.631 OD), when compared to the untreated cells (0.340 OD), indicated release of protein from *Listeria* cells treated with the bacteriocin of *P. pentosaceus* CFR SIII. This indicates that the bacteriocin disrupts the cell membrane causing leakage of intracellular protein, solutes and ions, affecting vital biochemical processes. However, release of nuclear material in treated cells was less and decrease in absorbance at 260 nm (0.253 OD) was observed, when compared to cells treated with pediocin preparation of same concentration from *P. acidilactici* K7 (0.636 OD) and enterocin from *Ent. faecium* MTCC 5153 (0.662 OD), which are known to affect cell death by pore formation in cell membrane. This indicated that the bacteriocin of *P. pentosaceus* CFR SIII exhibit drastic degradation of cytoplasmic constituents as seen in SEM of *P. acidilactici* B1153 treated with the same bacteriocin, wherein complete cell lysis was observed.

b) Effect of bacteriocin on glycolytic activity of *P. acidilactici* B 1153

The pH measurements of bacteriocin treated *P. acidilactici* B 1153 culture are presented in Fig. 3. The bacteriocin treated cultures showed alteration in pH similar to nisin and pediocin from *P. acidilactici* K7. The decrease in glycolytic rates as analyzed by concentration dependent drop in intracellular H^+ concentration has been reported in *Lactobacillus sake* and *Pediococcus pentosaceus* treated with bacteriocin pediocin PA-1 and nisin (Bennik et al. 1997). This drop in glycolysis rate lead to higher pH in the bacteriocin treated indicator cells in a time dependent manner compared to control. The lowering of glycolysis rate also reduces the ATP generation affecting several of energy dependent process such as active transport of solutes resulting in disruption of membrane potential leading to cell death.

Fig. 3

c) FTIR spectrum of bacteriocin treated *P. acidilactici* B1153

FTIR of whole microbial cells has been utilized as a reliable technique for microbiological analysis, including identification of microorganisms, study of microbial metabolism, antibiotic susceptibility, and other cell-drug interactions (Preisner et al. 2007). FTIR spectroscopy has been applied as a reliable method to study the putative mode of action of cell lytic bacteriocins from *Bacillus* sp. on *L. monocytogenes* (Motta et al. 2008). In the present study the FTIR spectroscopy was used to substantiate the cell lysis observed in SEM. The FTIR spectrum of bacteriocin treated *P. acidilactici* B1153 is shown in Fig. 4**.** In bacteriocin treated cells shift in absorbance in low frequency at 2957.5, 2934.7 and 2871.5 cm^{-1} was observed. The shift in absorbance band in the region of 3100-2800 cm^{-1} indicated that C-H anti symmetric and symmetric structural vibration of the lipid acyl chains. Also deformation in aliphatic, carbonyl group stretching and phosphate bond stretching, C-O-C deformations, which may include glycolipids, phosphodiester and polysaccharide, as revealed by decrease in spectra in the region of 1650-1055.8 cm^{-1}. Treated cells also showed frequency decrease in the range of 3000-3500 cm^{-1}, corresponding to NH_2 stretching. However, Motta et al. (2008) noticed frequency increase in 1,452 and 1,397 cm^{-1} and decrease in 1,217 and 1,058 cm^{-1}, corresponding assignments of fatty acids and phospholipids of *L. monocytogenes* cells treated with bacteriocin like substance of *Bacillus* sp.

Fig. 4

The primary mode of action of the bacteriocin PP-III was cell lysis. In the bacteriocin treated indicator organism loss of pH gradient as evidenced from SEM, changes in intracellular UV absorbing material and disruption of pH gradient were observed. This is in agreement with the previous reports on mode of action of bacteriocin produced by *Pediococcus,* wherein release of intracellular solute and subsequent imbalance in pH gradient and collapse of electron motive force was noticed (Bhunia et al. 1991; Christensen and Hulkins, 1992). Similarly, hydrophilic pore formation by pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0 (Chikindas et al. 1993) and by pediocin PD-1 produced by *Pediococcus damnosus* NCFB1832 (Bauer et al. 2005) in the cytoplasmic membrane of target cells by adhere nonspecifically to the surfaces of target cells there by inhibiting the transport of amino acids and cause the release of intracellular low-molecular-mass compounds, such as amino acids, ions and ATP was reported.

In conclusion, antimicrobial peptides, bacteriocins, produced by LAB represent unique antimicrobials with high diversity in their structure and physico-chemical properties. In the present study, a bacteriocinogenic LAB isolate, producing novel bacteriocin having broad spectrum of activity

against several pathogenic and spoilage bacteria, with distinctive physico-chemical properties, isolated from the natural ecological niche was identified. Keeping in view of the heat stability of the bacteriocin produced by the isolate, use of this culture or the bacteriocin produced by this isolate in minimally processed vegetables, where several saprophytic pathogens prevail, or in cheese preparation, can be emphasized as alternate to the use of chemical preservatives. Further characterization of the identified bacteriocin and technological evaluation of the isolate for preparation of fermented food products are under progress. The study indicates saprophytic LAB can be an ideal source for the study of new bacteriocins.

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Figure Captions:

Fig. 1 Tricine SDS-PAGE analysis of bacteriocin preparation. a) Silver staining of the gel and b) activity assay.

Lane 1, *P. pentosaceus* SIII; 2, P. acidilactici K7 (control). M is a low mol weight protein marker. Arrow indicates zone producing protein band s of around 5 kDa.

Fig. 2 SEM of *P. acidilactici* B1153 treated with bacteriocin from *P. pentosaceus C*FR SIII. Legends: 0 - Control, 1-1 h, 2 – 2 h of bacteriocin treatment

Fig. 3 Effect of bacteriocin on the glycolytic activity of cells of *P. acidilactici* B1153.

♦ no addition (control) and treated with bacteriocin produced by ■: *E. faecium* MTCC 5153, ▲: *P. acidilactici* K7, *: *P. pentosaceus* CFR SIII, and ×: Nisin (0.1mg ml $^{-1}$)

Fig. 4 FTIR analysis of bacteriocin PP- SIII treated *P. acidilactici* B1153.

A) The infrared spectra of untreated biomass; B) Cell mass treated with bacteriocin of *P. pentosaceus* CFR SIII; Circle indicates stretching of C-H & C=O groups upon treatment with bacteriocin.

Table 1 Antibacterial spectrum of *Pediococcus pentosaceus* CFR SIII

Indicator organism	Media/ Growth Condition	Antibacterial activity (AU ml^{-1})
Pathogenic bacteria		
Listeria monocytogenes Scott A	BHI/ 37°C, shaking	200
L. innocua		200
L. greyi		200
L. murrai		200
Salmonella paratyphi FB254		100
Staphylococcus aureus FB 298		100
Yersinia entericolitica MTCC 859		100
Salmonella typhi FB231		100
Lactic acid bacteria		
E. faecium MTCC 5153	MRS/37°C/ stationary	400
E. faecium DPC1146		400
Leuconostoc mesenteroides B640		100
Pediococcus acidilactici B1153		400
P. acidilactici K7		200
P. acidilactici PAC 1.0		200

Figure 1

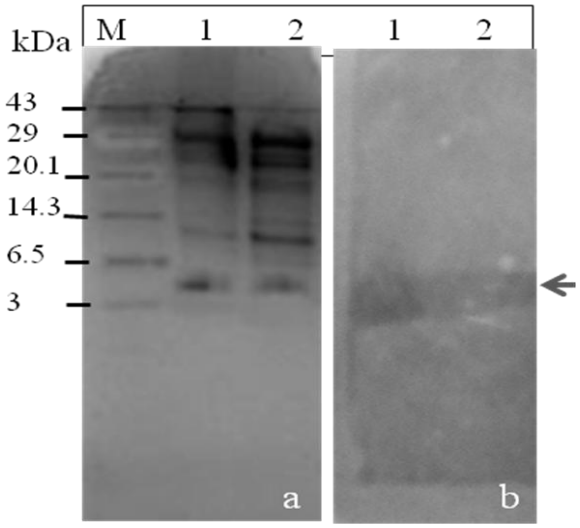


Figure 2

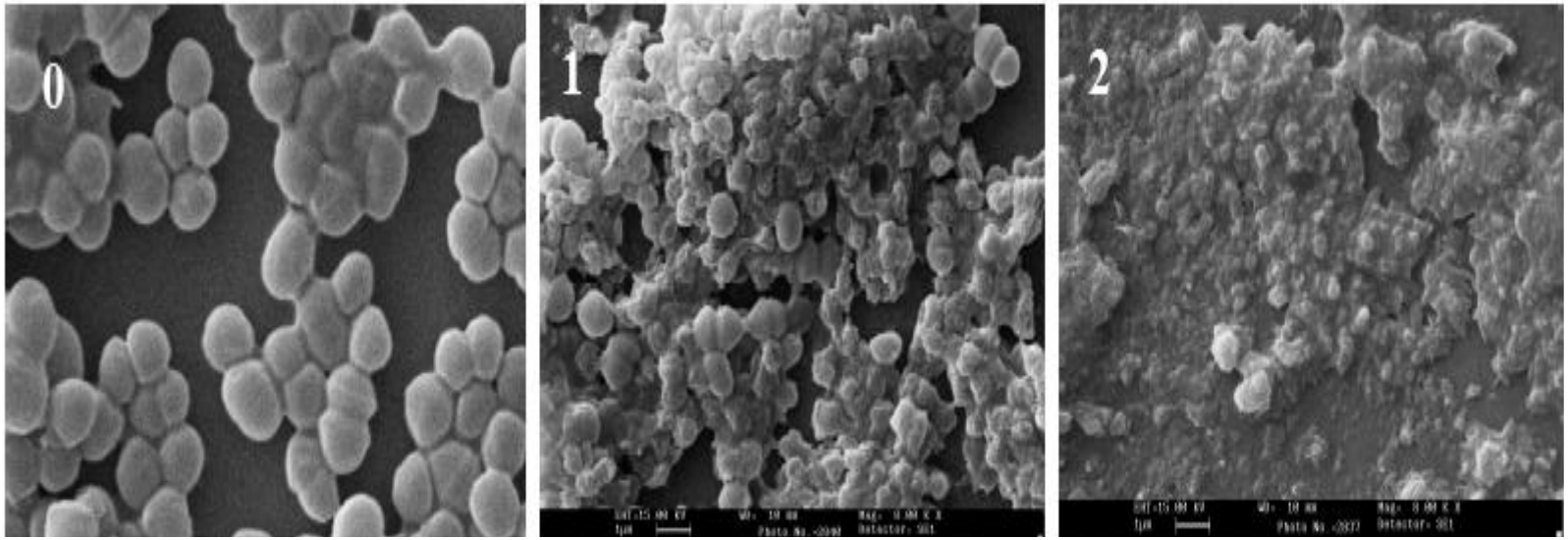


Figure 3

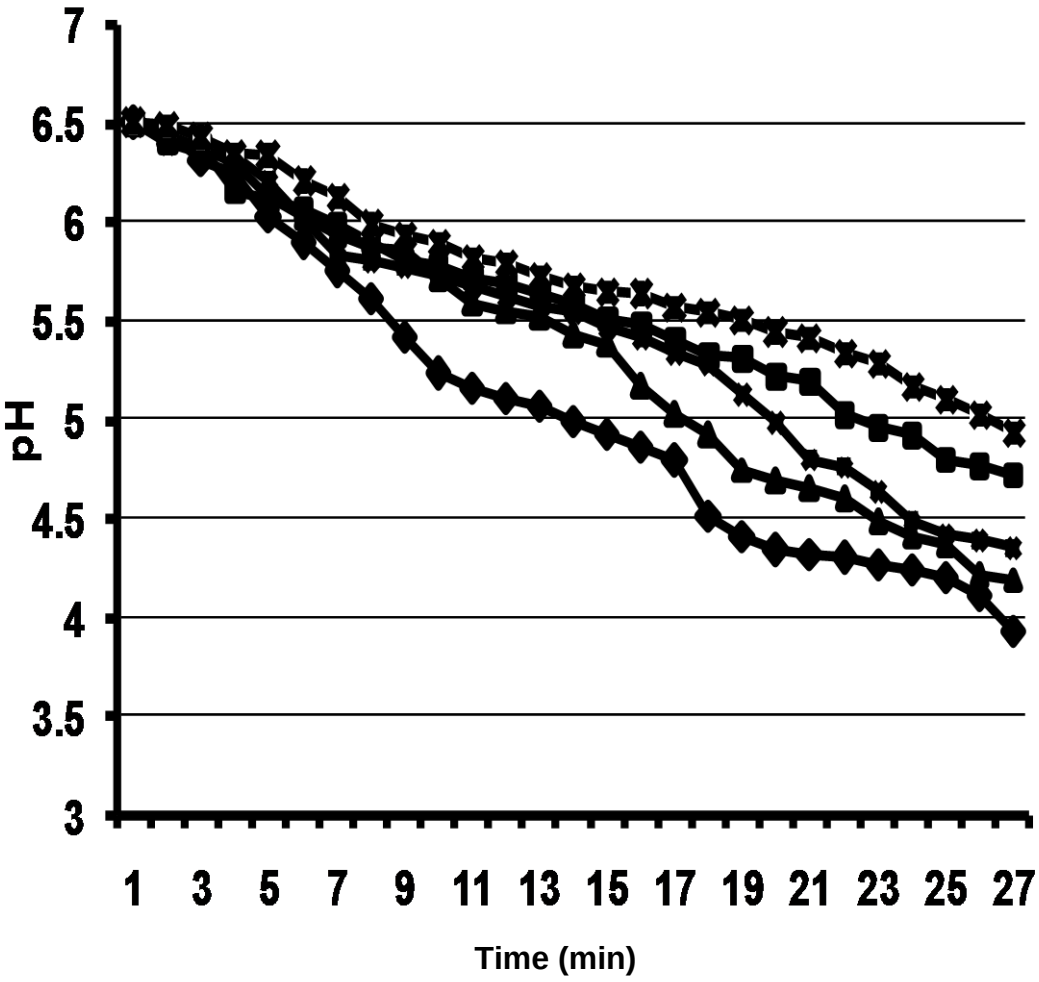
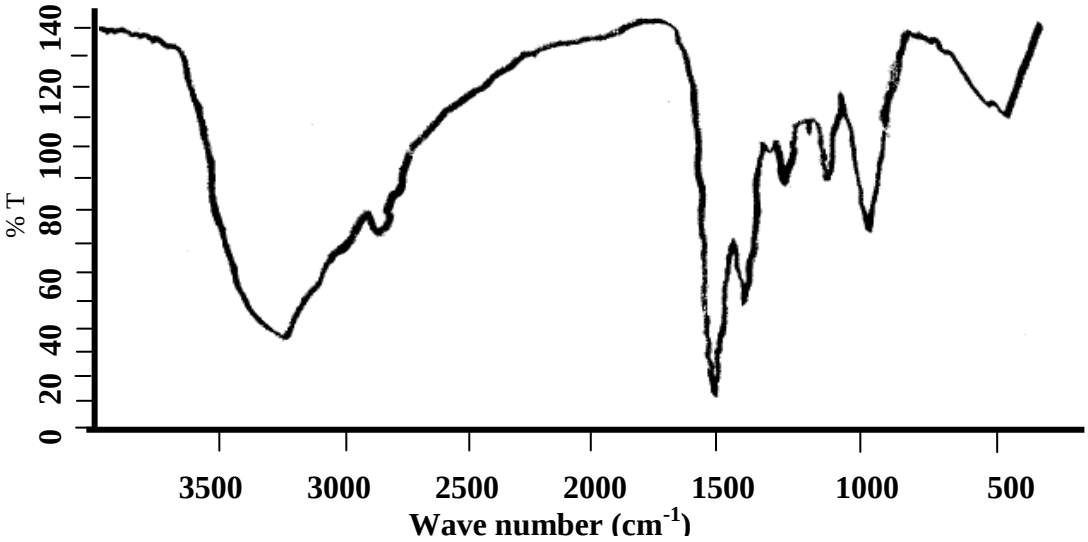


Figure 4

A)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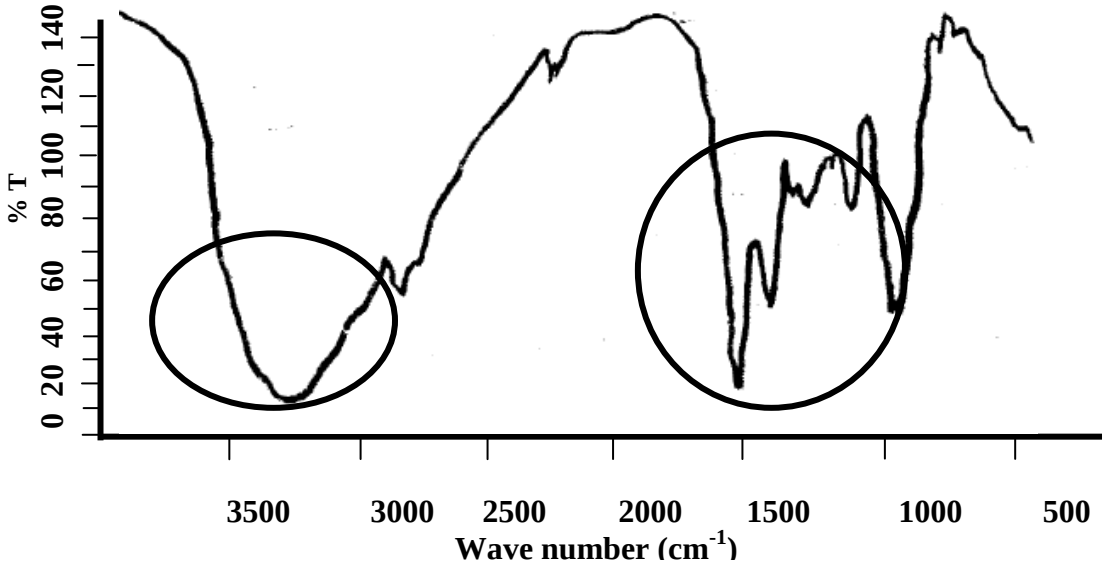
3500 3000 2500 2000 1500 1000 500
Wave number (cm-1)

B)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