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Characterization of a Bacteriocin Produced by *Pediococcus acidilactici* PBF and Its Plasmid Transfer by Electroporation

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***Pediococcus acidilactici* PBF, isolated from a commercial fermented sausage, produced a bacteriocin which is antagonistic towards several species of bacteria associated with food spoilage and health hazards of food origin. The bacteriocin was found to be sensitive to proteolytic enzymes and resistant to heat and organic solvents as well as active over a wide range of pH. Bacteriocin production was associated with two peptides, with molecular weights of 4.61 and 6.68 kDa and encoded by a 9.42 kbp plasmid. The preliminary results were suggested that the bacteriocin may be a two-peptide bacteriocin and these two peptides possess more antimicrobial activity together than on their own. In the main part of this investigation, the plasmid DNA responsible for bacteriocin production in *Pediococcus acidilactici* PBF, was transformed to *Pediococcus pentosaceus* Chr Hansen strain, which is a commercial starter culture used in the meat industry of Turkey. Bacteriocin phenotype was successfully electrotransformed to Bac$^-$ *Pediococcus pentosaceus* and this newly designed strain has now extra properties to be used in the food industry as a starter culture to enhance the nutritional quality and shelf life and also can be regarded as safe.**

Key words: *Pediococcus acidilactici*, Two peptide bacteriocin, Characterization, Electroporation.

In recent years, due to consumer demand, new functional foods have been developed. Many of these main food sources contain various chemical additives to improve the physical, chemical and microbiological properties of the foods and also to extend the shelf life of the product. Although some food chemical additives are approved by the FDA (Food and Drug Administration), recent studies have indicated that they may cause various problems for consumers health[1]. It is known that many of chemical preservatives lead to diseases such as cardiovascular problems or asthma in humans. However, some food additives are found unhealthy and have carcinogenic and toxic effects depending on the user rate. Due to such consequences, researches on natural and reliable additives are increased and researchers have tended to use natural and safe food biopreservatives such as bacteriocin, which is ribosomally-synthesized antimicrobial peptides and loosely defined as biologically active protein moieties[2]. Bacteriocin, determined as a healthy food biopreservative by WHO (World Health Organization) and considered as GRAS (Generally Recognized as Safe), is an alternative to chemical food additives with its colorless, tasteless and odorless properties as well as antimicrobial activity[2]. Bacteriocins produced by *Pediococcus* spp. belong to Class II, known as *anti*-listerial bacteriocins. ClassII bacteriocins such

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as others have thermal stability that ensures the availability in many foodstuffs like meat and dairy products, produced at high temperatures. In this group, pediocin AcH/PA-1, a 44 amino acid bacteriocin[3], produced by *P. acidilactici*, was the first thoroughly characterized and well-known bacteriocin[4].

It is very important to use bacteriocins in order to extend the shelf life of the food products. But, such systems need to be fully characterized before implementation to food industry. Physical and chemical properties of bacteriocin, mechanism of action, as well as antagonistic effects must be identified. Additionaly, bacteriocin has to be obtained in efficient amount. Thus, characterized and purified bacteriocin can be used as biological control agent in an economic way to preserve and increase the shelf life of food products[5]. However, new starter cultures can be designed to create a biological control system for extending the shelf life of food products and to have desired properties (good taste, flavor, softness/hardness of the product and anti-listerial bacteriocin production) in a healthy and reliable way with the use of plasmid analysis and advanced techniques, such as gene transfer and gene cloning. This study was undertaken with the purpose of characterization and purification of a bacteriocin produced by *P. acidilactici* PBF, and determination of the genetic basis of its production. The main part of this investigation was to transfer the plasmid DNA associated with bacteriocin production in *P. pentosaceus* Chr Hansen (Christian Hansen Laboratory, Horsholm, Denmark) strain, which is resistant to pediocin AcH and bacteriocin nonproducer (Bac^-) strain as well as a commercial starter culture used in the meat industry of Turkey.

MATERIALS AND METHODS

Bacterial cultures and cultivation

Pediococcus acidilactici PBF, isolated from commercial fermented sausage, was obtained from Ankara University, Microbial Genetic Laboratory Culture Collection (Turkey) and used as a bacteriocin producer strain in this investigation. The strain was grown in TGE (trypticase glucose yeast extract) medium at 35ºC for 18 h. The other indicator organisms used in this study, and their growth conditions are listed in table 1. Stock cultures were kept in broth medium with 25 % (v/v) sterile glycerol and stored at -80ºC.

Bacteriocin assay, antimicrobial spectrum and stability

Pediococcus acidilactici PBF was grown in TGE broth at 35ºC for 18 h and the cells were removed by centrifugation. Following the adjustment of the pH of cell free supernatant to the value of 7.0, supernatant was treated with catalase (1 mg/ml; Sigma Chem. Co., USA) and then heated for avoiding the other antagonistic effects such as low pH, hydrogen peroxide and bacteriophage[4]. After filtered sterilized (0.22 µm; Sartorius, Germany), cell free supernatant was used for all bacteriocin assays and called as crude bacteriocin extract. For activity assay, MRS (deMan Rogosa Sharpe, Merck, Darmstadt, Germany) soft agar seeded with *Lactobacillus sakei* NCDO 2714 as an indicator was overlaid to plates and crude extract was spot on lawn. Then plates were incubated overnight at 30ºC and examined for the presence or absence of an inhibition halo against *L. sakei* NCDO 2714 [4]. Antimicrobial spectrum was then determined with using the same technique against to other selected strains of both Gram-positive and Gram-negative bacteria as well as yeasts (Table 1) many of which are associated with food spoilage and food-related health hazards.

Crude extract was used in order to determine whether bacteriocin activity was affected after exposure to different enzymes, temperatures, pH values, organic solvents and protease inhibitors. Proteolytic and other enzymes (listed in table 2) were dissolved in sterile 4 mM phosphate buffer (1 mg/ml, pH 7.0) and treated with crude extract for 1h at 37ºC. The thermal stability of bacteriocin was tested by heating preparations at 80, 90 and 100ºC for 15 min, or by autoclaving at 121ºC for 15 min. To determine the antimicrobial activity at different pH values, the crude extract was adjusted to different pH values between the range of 2 to 12 with sterile NaOH or HCl. Organic solvents and other surfactants (Table 2) treated with crude extract at 1 % concentration and incubated at 37ºC for 5 h. After the all treatments mentioned above, the antimicrobial activity was checked using by spot on lawn method against to *L. sakei* NCDO 2714 [4]. Nontreated crude extract and solvents without bacteriocin were used as controls.

The quantitative antimicrobial activity of bacteriocin was determined by serial dilution (1:10-1:250) as described by Biswas *et al*.[6].

Influence of different parameters on bacteriocin production and bacteriocin production kinetic

In order to determine the factors which effective for maximum bacteriocin production, different parameters were evaluated. Primarily, optimum appropriate medium and incubation time were determined. An overnight culture of *P. acidilactici* PBF was separately inoculated to different mediums [TGE, TGE buffer (TGE including 5 % CH_3COONa and $C_6H_5Na_3O_7$, 0.05 % KH_2PO_4), Elliker (Merck, Darmstadt, Germany), M17 (Merck, Darmstadt, Germany), GM17 (M17 including 5 g/l glucose), MRS, BHI (Brain Hearth Infusion, Merck, Darmstadt, Germany)] with different initial pHs (5.0, 5.5, 6.0, 6.5, 6.8, 7.0, 7.5, 8.0, 9.0). All medium inoculations were incubated at different incubation temperatures (25ºC, 30ºC, 35ºC, 37ºC, 40ºC, 45ºC and 50ºC). To measure biomass by OD_{600}, as well as culture pH and bacteriocin activity unit (AU/ml), samples were taken in the beginning of inoculation (0 h) and at 2 h intervals during incubation[6,7].

Partial purification of bacteriocin and determination of its molecular weight in SDS-PAGE

Partial purification of the bacteriocin was carried out by the adsorption and desorption approach of Yang *et al*. [8]. After dialysis (Serva Spectra/pore MWCO 1000, USA) and freeze-drying process, tricine SDS-PAGE system was used to determine the molecular weight of bacteriocin. Two seperate gels were run together and one of the gels was used in determination the molecular size of bacteriocin while the other was used for antimicrobial activity. The gels were electrophoresed at 25 mA for 1 h, then 40 mA for 2 h with protein ladder (NOVEX Mark 12, Invitrogen, Finland) as a standard molecular mass marker. On completion of electrophoresis, one of the gels was stained with Coomasie Brillant Blue, and visualized. For activity assay, the other gel was washed with sterile water and then placed on a MRS agar prepoured plate, and overlaid with MRS soft agar seeded with *L. sakei* NCDO 2714. The plate was incubated at 30 ºC for overnight and examined for the position of the zone of growth inhibition by comparing with the other bands in the stained gel, then photopgraphed.

Location of gene responsible for bacteriocin production and its transfer by electroporation

Standard curing experiments were conducted to understand the location of bacteriocin coding gene. Elimination of bacteriocin activity was accomplished by growing *P. acidilactici* PBF strain in TGE broth containing increasing amounts of ethidium bromide (3 to 80 µg/mL) and acriflavine (3-25 pg/mL) with three consecutive transfers in a 24 h period, separately[9]. The cells were then diluted and cultured in pour plate. Following pour plating; the plates containing approximately 50 to 100 colonies were overlaid with MRS soft agar seeded with *Lb. sakei* NCDO 2714. Plates were further incubated overnight at 30ºC and examined for the absence of an inhibition halo against indicator bacteria which indicating phenotypic loss of the bacteriocin producing trait. These colonies were then named as bacteriocin-negative or bacteriocin-nonproducing (Bac^-) mutants. Totally, almost 50 colonies displaying Bac^- phenotype were picked randomly from the plates and their plasmids were compared with parental bacteriocin-positive or bacteriocin-producing (Bac^+) strain, PBF. *P. acidilactici* PAC1.0 (LMG 2002, Daeschel NC State) strain was used as a positive control for pediocin AcH/PA-1 producer in all experiments.

Plasmid DNAs of parental Bac^+ strain, PBF and Bac^- mutants, were extracted by using the commercial plasmid DNA isolation kit (Qiagen, Germany) and electrophoresed at 100 volt for 3 h, with using supercoiled DNA ladder (Invitrogen, Finland) as a standard molecular weight marker. On completion of the electrophoresis, the gel was visualized and plasmid profiles of parental Bac^+ strain, PBF and Bac^- mutants were compared. The plasmid responsible from bacteriocin production was purified from agarose gel by using the clean up gel extraction kit (NucleoSpin, Macherey-Nagel, Germany) and named as pPF1.0. Plasmid transfer to *P. pentosaceus* Chr Hansen, a Bac^- starter culture, was done with electroporation by using Gene Pulser electroporator (Bio Rad, USA). Cells were grown till the early exponential phase for OD 0.5-0.6 at 600 nm at 35ºC in 500 mL TGE broth supplemented with 40 mM DLthreonine. Then supernatant was removed after centrifugation at 4000 rpm for 10 min. The cells were washed twice

with electroporation buffer (5 mM potassium phosphate buffer [pH 7.4], 2 mM $MgCl_2$ in 25 % sucrose) and finally resuspended in ice-cold electroporation buffer and kept in ice. Purified pPF1.0 plasmid (1 µg/mL) in TE buffer (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA) and electrocompenent cells were mixed, then transfered to ice-chilled Gene Pulser cuvette (Bio-Rad Laboratories, Richmond, USA) on ice. A single pulse at the highest setting (2.5 kV, 25µF, and 200 ohms) was given to the plasmidless *P. pentosaceus*/purified pPF1.0 mixture. Cells were immediately transfered to a new tube containing TGE medium and incubated at 35ºC for 3-4 h without shaking. After incubation, the ability of the transformants to produce bacteriocin was detected on TGE agar plates, phenotypically as described above. Transformants were enumerated after 2 days incubation at 30ºC with examining the presence of an inhibition halo[10]. These colonies were picked up from the plates and examined for their plasmid profiles. The plasmid content of transformants was analyzed by agarose gel electrophoresis and compared with plasmid profiles of parental strain, PBF. Control experiments to determine the survival of the cells and the occurrence of mutants were performed by plating cells either that had received no electrical pulse or that were electroporated without plasmid DNA, on TGE media.

RESULTS AND DISCUSSION

Characterization of the bacteriocin produced by *Pediococcus acidilactici* PBF

In our study, bacteriocin produced by *P. acidilactici* PBF with an initial activity of 30.000 AU/ml, was found effective against a wide range of Gram-positive bacteria except *Leuconostoc lysis* Lys and *Lb. delbrueckii* subsp. *lactis* ATCC 10697, and presented *anti*-listerial activity by inhibiting *Listeria monocytogenes* (Table 1). No such activity was observed against to Gram-negative bacteria as well as yeasts, Table 1. To determine the nature of the antimicrobial compound of the PBF strain, retention of the activity of crude extract following several treatments was tested (Table 2). As a result, antimicrobial activity was lost following treatment with proteolytic enzymes such as trypsin, chymotrypsin, proteinase K; but maintained after autoclaving (121ºC for 15 min) and not affected by catalase, amylase as well as organic substances such as Tween 80 and SDS (Table 2). In the literature, all bacteriocins, produced by other *Pediococcus* strains, were inactivated by proteolytic treatment[11]. As a result, the loss of antimicrobial activity following treatment with proteolytic enzymes suggested that the active component secreted extracellularly by *P. acidilactici* PBF was proteinaceous in nature and could be bacteriocin. Retention of activity upon treatment with lipase, ribonuclease, lysozyme and other solvents indicated that the molecule is pure protein rather than conjugated one[12]. Additionaly, the crude extract of the PBF strain was also shown to be active at wide pH range 3-10. However, its activity was lost at pH above 10. As a result, the loss of activity at higher pH could be due to degradation of the molecule. Untreated samples showed no loss of activity. From this point of view, our results were found similar with other pediocins such as pediocin AcH/PA-1[13-16].

Bacteriocin production is greatly influenced by many parameters. From an economical perspective, determination of maximum bacteriocin production parameters such as medium ingredients, pH, incubation time and temperature is very important to reduce the costs of industrial-scale production. For our strain; maximum antimicrobial activity, 30.000 AU/mL, was recorded in TGE broth (pH 6.8), after 18 h incubation at 35ºC. No growth was observed in M17 and BHI mediums (data not shown). The rate of bacteriocin production (AU/ml) of *P. acidilactici* PBF began during the late stages of the exponential phase of the growth of the strain cultivated in TGE broth at 35ºC as indicated by an increase in OD_{600} and decrease in pH. The activity reached a maximum rate at 18 h during the late exponential and early stationary phase (Fig. 1). Several bacteriocins are produced in a maximum rate during the end of logarithmic growth phase or at the beginning of the stationary phase[17-19] genarally, pediocin production displays primary metabolite kinetics under lineer kinetic conditions with a peak activity paralleled the growth rate[20]. Similar to our results, when pediocin AcH from *P. acidilactici* H growth rate entered the stationary phase (18 hours), considerable amounts of pediocin were produced[3]. Also the factors influencing the production were

investigated and TGE broth was found more effective than MRS medium with resulting higher bacteriocin production.

Partial purification of the bacteriocin and determination of its molecular weight in SDS-PAGE

According to our SDS-PAGE gel assay results, partially purified bacteriocin of the strain contained a few protein bands (Fig. 2A, lane 1). Among these bands, zone of inhibition in activity gel (Fig. 2B, lane 1) was compared with molecular marker in stained gel (Fig. 2A, lane M) and two bands responsible for antimicrobial activity were shown tentatively linked to 6.6 and 4.6-kDa proteins. When bacteriocin preparation was treated with trypsin, the antimicrobial activity was seen to be lost in SDS-PAGE. Despite the structural similarities, bacteriocins molecular weight varies in Class IIa[11]. In this group, there are many bacteriocins in different range of molecular weight but, the bestknown is pediocin AcH/PA-1 from *P. acidilactici* PAC1.0, with molecular weight of 4.6-kDa [3,4]. Other characterized pediocins from *P. pentosaceus* such as pentocin L and S are about 27 and 25-kDa, respectively[21]. The antimicrobial activity of bacteriocins that have been studied so far is generally due to the action of a single peptide while some others rely on the complementary action of two peptides[22]. In the literature, there are many bacteriocins consist of two peptides belonging to Class IIb group[23], such as durancin L28-1A[24], enterocin L50[25], lactacin F[26], lactocin 705[27], lactococcin G [23], lactococcin Q [28], leucocin H [29], plantaricin NC8 [30]. Some of these two peptide bacteriocins cannot exert their antimicrobial activity as a single peptide independently, such as lactococcin G. However, two peptides together can possess much more activity when compare with each separated peptide activity[22].

Table 1. Antimicrobial spectrum of *Pediococcus acidilactici* PBF and growth conditions of indicator microorganisms used in this study (*: inhibition zone diameter as mm)

Indicator strains	Growth conditions	Antimicrobial activity (Inhibition zones*)
Bacillus cereus ATCC 9139 B	LB broth, 37ºC 18 h	4
Bacillus subtilis ATCC 21332	LB broth, 37ºC 18 h	6
Carnobacterium divergens NCDO 2306	MRS broth, 30ºC 18 h	9
Candida albicans ATCC 26555	TSB broth, 30ºC 18 h	-
Enterococcus casseliflavus NRLL 3502	MRS broth, 30ºC 18 h	15
Enterococcus faecalis ATCC 29212	MRS broth, 30ºC 18 h	17
Enterococcus faecium ATCC 6057	MRS broth, 30ºC 18 h	14
Escherichia coli ATCC 25922	LB broth, 37ºC 18 h	-
Lactobacillus delbrueckii subsp. *lactis* ATCC 10697	MRS broth, 30ºC 18 h	-
Lactobacillus plantarum NCDO 955	MRS broth, 30ºC 18 h	18
Lactobacillus plantarum DSM 20174	MRS broth, 30ºC 18 h	14
Lactobacillus sakei NCDO 2714	MRS broth, 30ºC 18 h	21
Lactococcus lactis ATCC 7962	GM17 broth, 30ºC 18 h	17
Leuconostoc lysis Lys	MRS broth, 30ºC 18 h	-
Leuconostoc mes. subsp. *dextranicum* NRLL 3469	MRS broth, 30ºC 18 h	17
Leuconostoc lactis NCDO 533	MRS broth, 30ºC 18 h	14
Listeria innocua M40	TSB broth, 35ºC 18 h	17
Listeria innocua B186/26B	TSB broth, 35ºC 18 h	11
Listeria monocytogenes ATCC 7644	TSB broth, 35ºC 18 h	18
Listeria monocytogenes EGDe	TSB broth, 35ºC 18 h	9
Micrococcus luteus M41	TSB broth, 30ºC 18 h	18
Pediococcus pentosaceus Chr Hansen (Bac-)	TGE broth, 35ºC 18 h	-
Pediococcus pentosaceus NCDO 992	TGE broth, 35ºC 18 h	11
Salmonella enterica serotype Typhimurium SL1344	LB broth, 37ºC 18 h	-
Staphylococcus aureus ATCC 6538	LB broth, 37ºC 18 h	12
Yersinia enterocolitica ATCC 9610	LB broth, 37ºC 18 h	-

To understand the mechanism of two peptides, complementary assays were carried out in this investigation. Two peptides, involved in bacteriocin activity was extracted from gel by using purify proteins from polyacrylamide gels kit (Thermo Scientific, USA) and mixed in 1:1 ratio together. Activity of each gel extract and their mixed solution was determined as described bacteriocin assay section. As a result, both peptides possessed antimicrobial activity on their own, but it was found most active when mixed together (data not shown). This result may be due to the complementary action of two peptides like enterocin L50 (EntL50), initially referred to as pediocin L50[25] and brochocin C[31]. Besides, it is not the first report about synergistic activity of two-peptide bacteriocins produced by *Pediococcus* strains. Our literature survey showed that, bacteriocin ST18 is the only two peptide pediocin produced by *P. pentosaceus* ST18, isolated from boza[16].

Table 2. Effect of pH, enzymes, organic solvents and heat on antimicrobial activity of *Pediococcus acidilactici* PBF

Treatments	Zone of growth inhibition*
Untreated	30.000 AU/ml
pH	at 3-10
Enzymes	
Amylase	+
Catalase	+
DNase	+
Lipase	+
Lysozyme	+
Ribonuclease A	+
Chymotrypsin	-
Trypsin	-
Pepsin	-
Proteinase K	-
Organic solvents	
Acetic Acid	+
Acetone	+
Acetonitrile	+
Chloroform	+
Ethanol	+
Isopropanol	+
Methanol	+
SDS	+
Triton X100	+
Tween 20	+
Tween 80	+
Urea	+
Heat	
80 ºC for 15 min	+
90 ºC for 15 min	+
100 ºC for 15 min	+
121 ºC for 15 min	+

(*: Clear inhibition zone (+) or no inhibition zone (-) against to *Lactobacillus sakei* NCDO 2714 using the agar spot test)

In our preliminary attempt, we here describe a two-peptide bacteriocin whose peptides act with their own selves but possess more activity together. However, these data need to be judged by reverse-phase chromatography, mass spectrometry, and amino acid sequencing to obtain certain results.

Location of genes responsible for bacteriocin production and its transfer by electroporation

Most of the Class IIa bacteriocins operons were shown to be located on plasmid although there are few reports stating the chromosomal location of bacteriocin genes[32-34]. Studies on *Pediococcus* strains emphasize that phenotype, responsible for bacteriocin production is encoded on plasmid DNA[35, 36]. However, it is indicated that size of the plasmids displays a wide variation between 8.35-19.35 kbp[37]. In our study, *P. acidilactici* PBF was shown to have 4 plasmids between the range of 5.99 kbp and 16.55 kbp on 0.7% agarose gel (Fig. 3, lane 1) and curing experiments were undertaken to determine whether or not any of these plasmids could carry the genetic determinants responsible for bacteriocin production phenotype in *P. acidilactici* PBF (Fig. 4A). Treatment of the strain with ethidium bromide and acriflavine was found effective in generating non-bacteriocinogenic derivatives (Fig. 4B). Such cells were considered as bacteriocin-nonproducing (Bac^-) mutants. Bac^- cells were then used along with the Bac^+ parental *P. acidilactici* PBF strain on agarose gel electrophoresis to compare their plasmid profiles and to assign the bacteriocin production trait to a specific plasmid (Fig. 3, line 1-2). The results of this comparison showed that the loss of the plasmid, which is in 9.42 kbp molecular weight, resulted in a detectable phenotypic change such as loss of bacteriocin production in the strain and this situation has suggested and implicated the possibility of plasmid DNA location of this genetic determinant. The gene responsible for bacteriocin production was found to be encoded

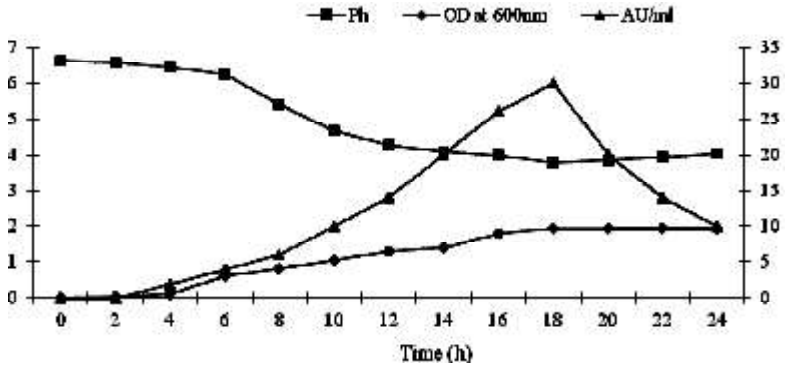

Fig. 1. The rate of bacteriocin production (AU/ml X 10^3) in relation to cell growth (OD at 600_{nm}) and acid production (reduction in pH) by *Pediococcus acidilactici* PBF in TGE broth at 35 ºC

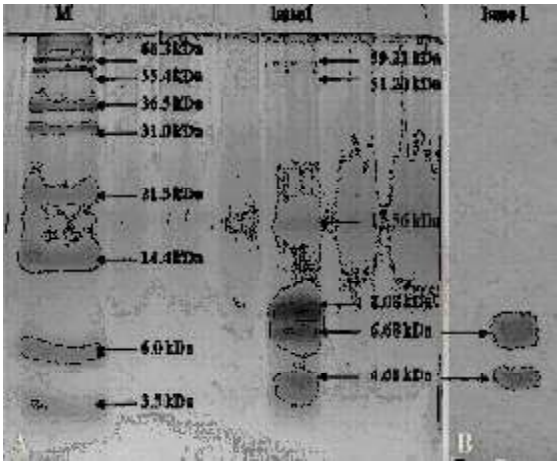

Fig. 2. Coomassie-Brillant Blue stained SDS-PAGE gel: M contains molecular weight standard (NOVEX Mark 12, Invitrogen, Finland), Lane 1 contains partially purified bacteriocin produced by *Pediococcus acidilactici* PBF (A); Activity assay gel: Lane 1 contains bacteriocin showing zones of growth inhibition corresponding to two bands of molecular weight, approximately 4.61 and 6.68-kDa (›) (B)

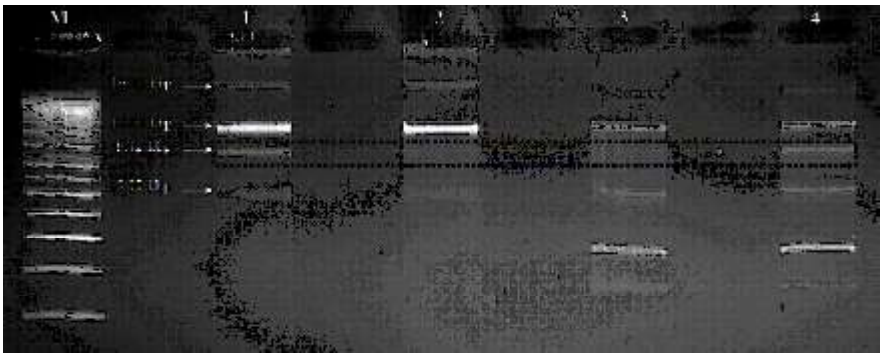

Fig. 3. Plasmid profiles of the strains on agarose gel: M contains molecular weight standard (supercoiled DNA ladder, Invitrogen, Finland), Lane 1 contains plasmids of *Pediococcus acidilactici* PBF (parental Bac^+ strain), Lane 2 contains plasmids of *Pediococcus acidilactici* PBF after curing experiment (Bac^- mutant), Lane 3 contains plasmids of *Pediococcus pentosaceus* Chr Hansen (Bac^- recipient), Lane 4 contains plasmids of *Pediococcus pentosaceus* Chr Hansen (Bac^+ recombinant) after transfer the pPF1.0 plasmid (9.42-kbp) by electroporation

in a plasmid DNA with an approximate molecular weight of 9.42 kbp.

In recent years, many studies on cloning the plasmids, linked to bacteriocin production, were carried out[38]. Pediocin PA-1 has been cloned and expressed in different LAB strains[40,41], *E. coli*[39] and baker's yeast *Saccharomyces cerevisiae*[42]. In our study, plasmid DNA, which was found to associate with bacteriocin production with a molecular weight of 9.42 kbp, was gel purified and transformed into a Bac^- recipient of commercial starter strain (Fig. 4C), *P. pentosaceus* Chr Hansen. Upon transformation by electroporation, cells of Bac^- *P. pentosaceus* Chr Hansen were shown to reproduce and/or reexpress bacteriocin as indicated by the presence of growth inhibition of *L. sakei* NCDO 2714 around some colonies (Fig. 4D). The Bac^+ recombinant colonies were purified and shown to carry plasmid DNA associated with bacteriocin production when compared with Bac^- *P. pentosaceus* Chr Hansen, as shown by agarose gel electrophoresis (Fig. 3, line 3-4). Nevertheless, bacteriocin produced by recombinant colonies with an initial activity of 14.000 AU/ml, was found effective against *L. monocytogenes* and lost its activity following treatment with proteinase.

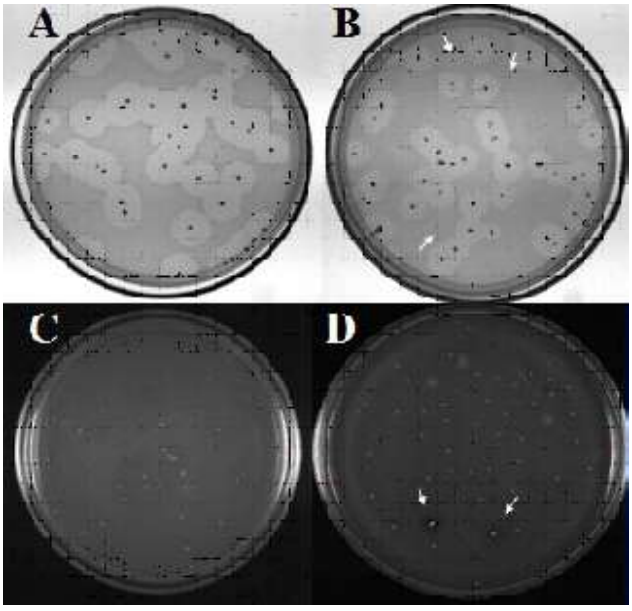

Fig. 4. Presence or absence of an inhibition halo against *Lactobacillus sakei* NCDO 2714 around colonies: Bac^+ *Pediococcus acidilactici* PBF (A), Bac^- mutant *Pediococcus acidilactici* PBF after curing experiment (B), Bac^- recipient *Pediococcus pentosaceus* Chr Hansen (C), Bac^+ recombinant-transformant *Pediococcus pentosaceus* Chr Hansen (D)

CONCLUSION

In conclusion; *P. acidilactici* PBF, isolated from commercial fermented sausage, produced an antimicrobial peptide which is in protein nature and have an inhibitory activity to a variety of spoilage and pathogenic microorganisms. Additionally, Bac^+ phenotype was successfully electrotransformed to commercial starter strain of Bac^- *P. pentosaceus* Chr Hansen. This designed strain has now extra properties to be used in food industry as a starter culture to enhance the nutritional quality as well as a potential biological barrier/ biopresevative to prevent the growth of spoilage bacteria and foodborne pathogens associated with foods such as *L. monocytogenes*. Also the purified plasmid responsible for bacteriocin production, could be a valuable candidate for the construction of recombinant plasmid, and thus, for future strain development and genetic engineering studies of *P. pentosaceus* Chr Hansen as well.

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