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Screening and identification of bacteriocin-like inhibitory substances producing lactic acid bacteria from fermented products

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Abstract

A total of 544 isolates of lactic acid bacteria isolated from fermented products: 451 isolates from fermented products from Thailand and 93 isolated from French fermented products were screened for bacteriocin production. 10 isolates showed antimicrobial activities. Identification of the selected strains by random amplification of polymorphic DNA-polymerase change reaction using the primers E1, E2 and M13 showed three different RAPD patterns. One of the isolates from each product was chosen and good antimicrobial activity were displayed by 3 strains including MP6/2, PKS2-1 and OV1-1 producing in MRS broth bacteriocin-like substance (BLIS) inhibiting *Candida albicans* ATCC 90028, *Carnobacterium maltraromaticum* NCDO 2760, *Lactobacillus sakei* subsp. *sakei* JCM 1157, *Listeria innocula* CIP 80.11, *Listeria ivanovii* SLCC 2379, *Pseudomonasc aeruginosa* ATCC 27853, *Staphylococcus aureus* CIP 76.25 and *Streptococcus mutans* used as indicator strains. However, inhibitory activities of MP6/2 and PKS2-1 were related to the production of either organic acid, while OV2-1 was not affected by heating. The inhibitory activities of these three isolates were sensitive to proteolytic enzymes. 16S rDNA gene sequencing allowed to identify the MP6/2 as *Pediococcus pentosaceus* while PKS2-1 and OV1-1 were identified as *Enterococcus faecium*. It was concluded that the obtained isolates may be used as bio-preservative cultures to produce fermented foods.

Keywords: lactic acid bacteria; bacteriocin; fermented food products; antimicrobial.

Practical Application: The well-characterized bacteriocin-like substance producing lactic acid bacterial strains can be used for the production of functional fermented foods.

1 Introduction

Nowadays consumers are aware of the composition of their foods and want to avoid chemical preservatives and taste enhancers like monosodium glutamate. The consumer demands and government regulations push the food manufacturers to find the natural preservatives to control the contaminating microbes, mostly responsible for food spoilage and poisoning. Biopreservation - efficient and natural way of preserving the foods, is defined as employing the non-pathogenic, beneficial bacteria or their secondary metabolites to extend the shelf-life of foods and to increase the microbiological safety.

Lactic acid bacteria (LAB) are Gram-positive rods or cocci, aerotolerant, homofermentative or heterofermentative and lactic acid producers. LAB can produce organic substances that are responsible for the unique aroma (Caplice & Fitzgerald, 1999). *Lactobacillus*, *Lactococcus*, *Carnobacterium*, *Oenococcus*, *Melissococcus*, *Pediococcus*, *Enterococcus*, *Leuconostoc*, *Lactosphaera*, *Streptococcus*, *Tetragenococcus*, *Weissella*, and *Vagococcus* are the common genera of the LAB. The LAB is found in fermented products, beverages, milk and even in meat products, are purposely used to prevent the food spoilage and to enhance the quality of the foods like improved flavor and texture. Most of the known LAB strains are represented by the genus *Lactobacillus*, and many of the strains are considered as probiotics (Parada et al., 2007). The LAB is safe and can be used as competitive starter cultures for the controlled fermentation processes (Cintas et al., 2001). Some of the LAB strains produce antagonistic substances when competing with other microbes in the surroundings. These substances are called bacteriocins or bacteriocin-like inhibition substances (BLIS) (Moreno et al., 2000).

Bacteriocins are ribosomally synthesized, small (<10 kDa), bactericidal, heat-stable, antibiotic-like substances, amphiphilic peptides or protein in nature, produced by the LAB. Bacteriocins are primarily defined in *Escherichia coli* with the name of colicins. Colicins can also kill the same strain or closely related bacteria (Cascales et al., 2007).

Bacteriocins are classified into three classes as following; Class I or lantibiotics, are peptide substances that contain unsaturated amino acids (2- aminoisobutyric acid and dehydroalanine), and polycyclic thioether amino acids (methyllanthionine

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or lanthionine), Class II or non-lantibiotics, are small, heat stable, non-lanthionine containing membrane peptides. Non-lantibiotics are further separated into two subclasses such as Subclass II a (listeria active bacteriocins or pediocin-like), and Subclass II b (two-component bacteriocins), Class III or bacteriocins, large (>30 kDa) and heat sensitive. Lactoccin G, Lactococcin MN, Nisin, Leucocin H, Plantaricin W, Plantaricin EF, Plantaricin S, Plantaricin JK, Lactacin F, and Lactocin 705 are common bacteriocins produced by the LAB (Zacharof & Lovitt, 2012).

LAB and bacteriocins are considered as safe and are used as biopreservatives. Several reports proved that the fermented foods (FF) are a cheap and major source of bioactive LAB strains. Many LAB strains isolated from FF are used further as the starters for the preparation of high quality functional FF (Woraharn et al., 2014, 2015). The importance and probiotic nature of LAB in FF have been reported (Angmo et al., 2016; Rhee et al., 2011). Further studies are needed to screen and to identify more commercially important LAB strains that are able to produce BLIS. Thus, the aim of the current study was the screening and the identification of bacteriocin-producing LAB strains from FF of Thailand and France with the potential to be applied in FF production.

2 Materials and methods

2.1 Isolation of Lactic Acid Bacteria (LAB)

The fermented food (FF) samples were collected from markets in Nantes, France and in Chiang Mai, Northern Thailand. LAB strains were isolated by serial dilution and plating method as described earlier by Woraharn et al. (2014) and stored at -20 °C with 20-25% glycerol as cryoprotectant.

2.2 Strains and culture condition

LAB isolates and *Lactobacillus sakei* subsp. *sakei* JCM 1157 were maintained at 37 °C in de Man and Rogosa Sharpe (MRS) media (Himedia, India). The bacterial indicator strains such as *L. sakei* subsp. *sakei* JCM 1157 and *Listeria ivanovii* SLCC 2379 were obtained from Institut National de la Recherche Agronomique (INRA), Nantes, France. *Brochothrix thermosphacta* DSMZ 20171, *Carnobacterium maltaromaticum* NCDO 2760, *Staphylococcus aureus* CIP 7625, *Escherichia coli* CIP 76.24, and *Listeria innocua* CIP 80.11 were obtained from Ecole Nationale Nantes Atlantique Vétérinaire, Agroalimentaire et de l'Alimentation (ONIRIS), Nantes, France. *Cronobacter sakazakii* ATCC BAA-894, *E. coli* ATCC 5922, *Salmonella enterica* serovar Typhi (*Salmonella* Typhi), *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei*, *S. aureus* ATCC 5923, *Streptococcus mutans*, and *Bacillus cereus* ATCC 11778 were obtained from Facuty of Pharmacy, Chiang Mai University (CMU), Thailand. The indicator strains were sustained in brain heart infusion (BHI) media (Himedia, India). *Candida albicans* ATCC 90028 was obtained from CMU. Yeast was grown in sabouraud dextrose media (Himedia, India).

2.3 Assessment of antimicrobial activity

Agar overlay and agar well diffusion methods were employed as described previously with slight modifications (Birri et al., 2013; Hwanhlem et al., 2014; Sirilun et al., 2017). Briefly, an overnight culture of LAB isolates was stabbed on MRS agar (1.5% agar) plate and incubated at 37 °C for 3 hr. Then, soft agar (0.8% agar) containing 10^6 CFU/mL indicator strain was overlaid on the plates, and plates were incubated at 37 °C for 24 hr. The formation of clear inhibition zones surrounding the colonies indicated antimicrobial activity.

The cell-free supernatant (CFS) of 24 hr culture of LAB isolates was used for agar well diffusion method as detailed (Hwanhlem et al., 2014; Sirilun et al., 2017).

CFS of positive LAB isolates were treated with catalase (Sigma Aldrich, St. Louis, USA; 1 mg/mL) to remove the hydrogen peroxide content and proteinase K (Sigma Aldrich, St. Louis, USA; 1 mg/mL) for 1 hr 37 °C. The treated CFS samples were further analyzed for the antimicrobial activity by agar well diffusion assay.

2.4 Characterization of Bacteriocin-Like Inhibitory Substances (BLIS)-producing LAB isolates

BLIS-producing LAB isolates were subjected to Gram staining, and microscopic analysis to check the cell wall pattern, and their shape. Then, the isolates were assayed for catalase activity. The catalase-negative and Gram-positive isolates were further tested for their ability to grow in MRS broth at 45 °C with 6.5% NaCl and pH 9.6.

2.5 Random Amplification of Polymorphic DNA (RAPD)

The selected LAB strains were subjected to RAPD analysis to differentiate the strains using E1, E2 (Kirilov et al., 2011) and M13 (Rossetti & Giraffa, 2005) primers (Supplementary Material, Table S1). Total DNA was extracted from LAB isolates using commercial bacterial DNA extraction kit following the manufactures instructions (E.Z.N.A.® Bacterial DNA Kit, D3350-01, Omega BioTek, USA) and the A260/A280 ratio of DNA samples was determined using a spectrophotometer (BioPhotometer, Eppendorf 6131, USA). PCR reaction was carried out using RAPD primers by following the previously described conditions (Kirilov et al., 2011; Rossetti & Giraffa, 2005). The amplifications were performed in a Touchgene gradient thermocycler (Techne, UK). The PCR amplicons were separated by electrophoresis at 70 V on 1.5% (w/v) agarose gels with ethidium bromide (0.5 µg/mL), in 0.5X TAE buffer and the band pattern was observed under UV light.

2.6 16S rDNA gene sequencing

The representative LAB strains from each RAPD profiled groups were subjected to genomic DNA isolation and 16S rDNA amplification using universal primers (fD1 and rD1; Table S1) (Weisburg et al., 1991). The sequencing of PCR amplicons was done by MilleGen sequencing service (Labège, France). The attained sequences were BLAST with other sequences, and a phylogenetic tree was generated.

2.7 Effect of heat on bacteriocin activity

The selected LAB strains were cultured in MRS broth at 37 °C for 16-24 hr, and CFS was collected and filtered through a membrane filter (0.45 µm). The filtered CFS was neutralized

(pH 7.0) by adding sterile 12 N sodium hydroxide, and incubated at 100 °C for 0, 10, 15, 30 and 60 min, and 121 °C for 15 min. After incubation, CFS samples were assayed for residual bacteriocin activity against *L. sakei* subsp. *sakei* JCM1157 by agar well diffusion assay (Hwanhlem et al., 2014).

CFS samples were diluted two-fold in 20 mM phosphate buffer and aliquots of 50 µL from each dilution were tested against indicator strains by well diffusion method. The activity was represented in arbitrary units per milliliter (AU/mL) (H-Kittikun et al., 2015) (Equation 1).

$$AU / mL = \frac{1000}{V} D \quad (1)$$

where: V is the volume of CFS and D is the dilution factor.

2.8 Effect of enzymes on bacteriocin activity

The neutralized filtered CFS was treated with enzymes (catalase, proteinase K, α-chymotrypsin, and trypsin) at 37 °C for 4 hr, after incubation, the pH of the solution were neutralized with 100 mM sterile potassium phosphate buffer and the reaction was arrested by heat (H-Kittikun et al., 2015). Bacteriocin activity of the samples was assayed against *L. sakei* subsp. *sakei* JCM 1157 by agar well diffusion method.

2.9 Statistical analysis

All assays were performed in triplicates. The data are stated as the mean ± standard deviation (SD). Statistical analysis was achieved using SPSS software version 17.0. The data were subjected to one-way analysis of variance (ANOVA) to determine whether there were any differences between the antibacterial activities of samples and control. Significant differences were identified by Duncan test on the level of $P < 0.05$.

3 Results

3.1 Isolation of BLIS producing LAB

A total of 544 LAB isolates (451 from fermented products from Thai, and 93 from fermented products from France) were isolated from FF (Table S2). The strains were assessed for production of antimicrobial substances by agar overlay method, the occurrence of clear inhibition zones around the colonies indicates that particular LAB colonies can produce some active substance capable of inhibiting the growth of indicator strains, and about 26 isolates were selected with antimicrobial activity (Table S2, and Figure 1A). The cell-free supernatant (CFS) of selected 26 LAB isolates were neutralized (pH 6.5-7.5) and assayed by agar well diffusion method, and ten isolates that are found to be most active were selected for further studies (Figure 1B).

The selected ten isolates (MP6/2, PKS2-1, PKS2-2, PKS3-7, PKS3-8, PKS4-2, OV1-1, OV2-3, OV3-4, and OV3-6) showed antimicrobial activity against indicator strains such as *B. cereus*, *C. maltaromaticum* NCDO 2760, *L. sakei* subsp. *sakei* JCM 1157, *L. innocua* CIP 80.11, *L. ivanovii* SLCC 2379, *S. aureus* CIP 76.25, *S. mutans,* and *C. albicans* ATCC 90028 (Table 1). To verify the presence of BLIS in CFS, neutralized CFS was prepared with catalase (to remove hydrogen peroxide) and treated with proteinase K. The treated CFS was tested against *L. innocua, L. ivanovii,* and *L. sakei* subsp. *sakei* JCM 1157 and found that proteinase K treatment inactivates the inhibitory activity of CFS (Table 2).

3.2 Characterization and identification of BLIS-producing LAB isolates

The morphological observation and physiological properties of 10 isolates were determined. All 10 isolates were Gram-positive, cocci, catalase negative, and could grow at 45 °C in the presence of 6.5% of NaCl at pH 9.6 (Table S3). The isolated strains were exposed to RAPD analysis using three different primers

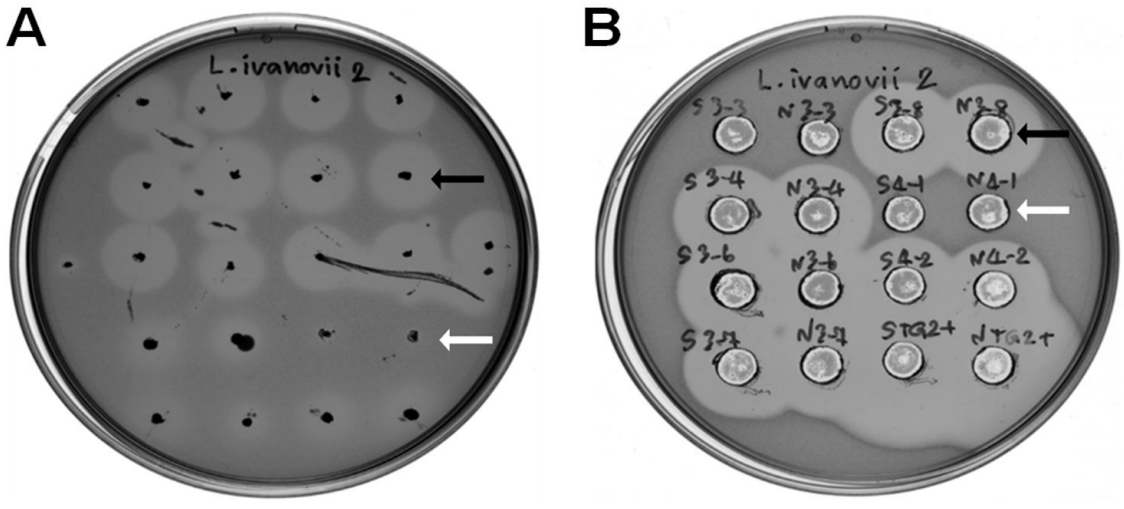

Figure 1. Detection of bacteriocin–like inhibitory substances (BLIS) producing ability of isolated lactic acid bacteria by agar overlay method (A) and agar well diffusion assay (B). The black and white arrows indicate examples of positive and negative result with or without a clear halo zone, respectively.

Table 1. Antimicrobial activity of isolated lactic acid bacteria revealed by agar well diffusion assay.

Indicator strains	Antimicrobial activity									
	MP6/2	PKS2-1	PKS2-2	PKS3-7	PKS3-8	PKS4-2	OV1-1	OV2-3	OV3-4	OV3-6
Bacillus cereus ATCC 11778	+	+	+	+	+	+	+	+	+	+
Brochothrix thermosphacta DSMZ 20171	-	-	-	-	-	-	-	-	-	-
Carnobacterium maltraromaticum NCDO 2760	+	+	+	+	+	+	+	+	+	+
Cronobacter sakazakii ATCC BAA-894	-	-	-	-	-	-	-	-	-	+
Lactobacillus sakei subsp. *sakei* JCM 1157	+	+	+	+	+	+	+	+	+	+
Listeria innocua CIP 80.11	+	+	+	+	+	+	+	+	+	+
Listeria. ivanovii SLCC 2379	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus ATCC 5923	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus CIP 76.25	+	+	+	+	+	+	+	+	+	+
Streptococcus mutans	-	+	-	-	-	-	+	-	-	-
Escherichia coli ATCC 5922	-	-	-	-	-	-	-	-	-	-
Escherichia coli CIP 76.24	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	+	+	-	+	-	+	+
Salmonella enterica serovar Typhi	-	-	-	-	-	-	-	-	-	-
Shigella sonei	-	-	-	-	-	-	-	-	-	-
Candida albicans ATCC 90028	-	+	+	+	+	+	+	-	+	-

−: no inhibition; +: presence ≥2 mm. diameter of clear inhibition zones.

Table 2. Bacteriocin–like inhibitory substances producing ability of the isolates revealed by agar well diffusion assay.

Isolates	Antimicrobial activity (CFS/K)		
	L. innocua	*L. ivanovii*	*L. sakei* subsp. *sakei* JCM1157
MP6/2	+/-	+++/-	+/-
PKS2-1	+/-	+++/-	++/-
PKS2-2	+/-	+++/-	++/-
PKS3-7	+/-	+++/-	++/-
PKS3-8	+/-	+++/-	++/-
PKS4-2	+/-	+++/-	++/-
OV1-1	+/-	+++/-	++/-
OV2-3	+/-	+++/-	++/-
OV3-4	+/-	+++/-	++/-
OV3-6	+/-	+++/-	++/-

Cell free supernatant (CFS). CFS treated with proteinase K (K). No inhibition (-). ≤1 to <5 mm diameter of inhibition zone (+). ≤5 to <10 mm diameter of inhibition zone (++). ≤ 10 mm diameter of inhibition zone (+++).

(M13, E1, and E2), and clear, reproducible amplification patterns were observed (Figure 2). Three different clonal groups were detected based on the RAPD patterns. MP6/2 showed amplification with M13 primer, but no amplification was observed with E1, and E2 primers (clonal group 1). The isolates PKS2-1, PKS2-2, PKS3-7, PKS3-8, and PKS4-2 produced a different pattern (clonal group 2). The isolates OV1-1, OV2-3, OV3-4, and OV 3-6 showed a unique pattern of amplification (clonal group 3) (Table S4).

Three isolates (OV1-1, PKS2-1, and MP6/2), one from each clonal group were selected for molecular identification by partial 16s rDNA gene sequencing. A fragment of (~450 bp) 16s rDNA was amplified and sequenced. The obtained sequences of OV1-1, PKS2-1, and MP6/2 were compared with the GenBank database using the BLAST. Dendrogram construction of the relationship between the 16S rDNA gene sequences of three isolates was done by the phylogenetic analysis web server (Phylogeny.fr, 2018) (Figure 3). The comparative analysis revealed that the strain MP6/2 from fermented products from Thailand was most similar to *Pediococcus pentosaceus,* and strain OV1-1 and PKS2-1 from fermented products from France and Thailand, respectively were identified as *Enterococcus faecium*.

3.3 Effect of heat and enzymes on bacteriocin activity

The heat treatment at 100 °C for 30 min reduced the bacteriocin activity of MP6/2 up to 50%. 60 min treatment nullified the activity entirely, while OV1-1 and PKS2-1 conserved 25, and 50% of activity even after 60 min of heat treatment. The heating at 121 °C for fifteen minutes inactivated the MP6/2 activity, whereas OV1-1 and PKS2-1 retained 50% and 12.5% of activity after the treatment at 121 °C for fifteen minutes (Table S5).

Bacteriocin activity of CFS of MP6/2, OV1-1, and PKS2-1 was stable after heating at 100 °C for ten minutes. However, the activity of MP6/2 and PKS2-1 were decreased by 50% after neutralization. Catalase treatment has not affected the activity of isolates. α-chymotrypsin, proteinase K, and trypsin treatments completely nullified the activity of MP6/2, OV1-1, and PKS2-1 (Table S3).

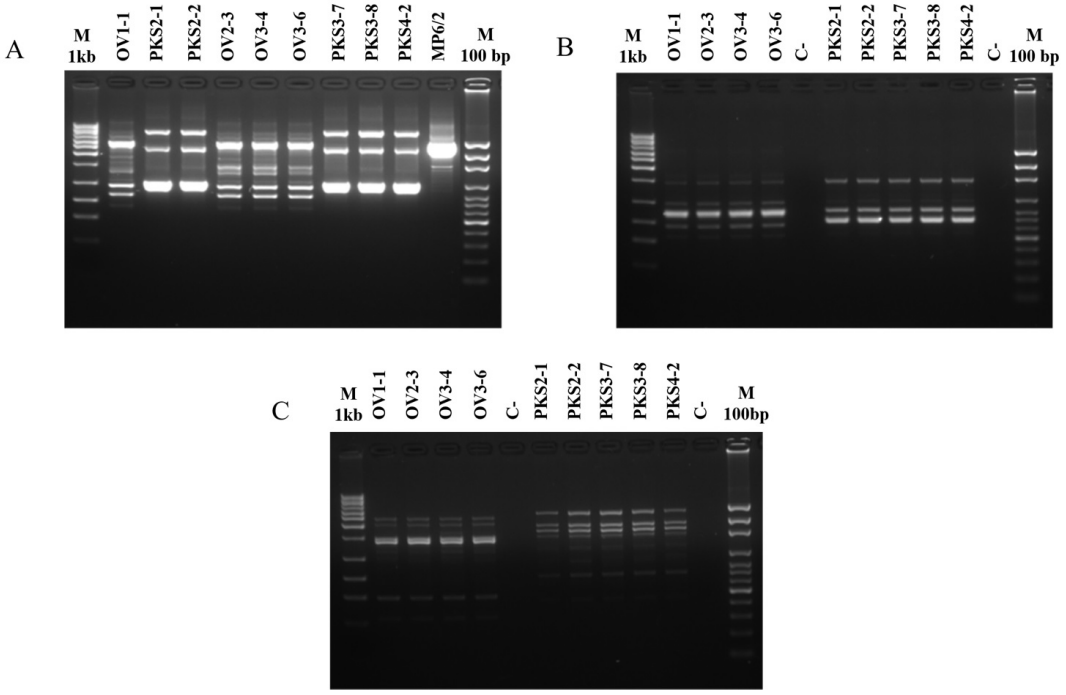

Figure 2. Random amplification of polymorphic DNA-polymerase change reaction (RAPD-PCR) fingerprinting pattern of BLIS-producing LAB isolates with primer M13 (A), primer E1 (B) and primer E2 (C). Lanes M: 100 and 1000 bp ladders.

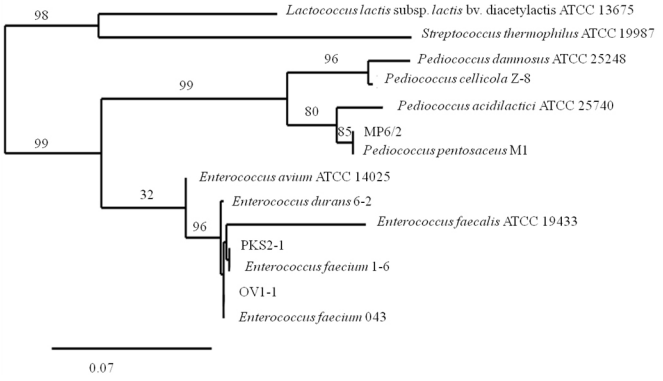

Figure 3. Phylogenetic tree showing the relationship between the 16S rDNA gene sequences of OV1-1, PKS2-1 and MP6/2. The bar indicates 0.07 substitutions per nucleotide position.

4 Discussion

About 544 LAB isolates were obtained in primary isolation process, in which many strains were acquired from fermented products from Thailand (451 isolates) and from France (93 isolates). The results indicate that FF from Thailand are abundant in LAB strains. The initial screening by agar overlay results showed that only 11 strains of Thai origin and 15 strains of French origin could produce antimicrobial substances. The CFS of all 26 isolates were neutralized (pH 6.5-7.5), neutralization helped to avoid the positive results from the presence of organic acids in CFS. The obtained results showed that only ten isolates can able to produce BLIS (Table S2).

Further study confirmed that the selected ten isolates could hinder the growth of Gram-positive bacteria more effectively than that of the Gram-negative strains, while some isolates showed inhibition against *C. albicans* (Table 1).

The results suggested that BLIS of selected strains were active against genetically close groups of bacteria. The same scenario was reported previously, *E. faecium* AQ71 was active against Gram-positive bacteria but not against Gram-negative bacteria like *Salmonella* spp. and *E. coli* (Ahmadova et al., 2013). Aspri et al. (2017) reported a spectrum of inhibitory activity of bacteriocin from *E. faecium* against Gram-positive bacteria. Whereas, bacteriocin CAMT2 produced by *Bacillus amyloliquefaciens* displayed antimicrobial activity against both Gram-positive and negative bacteria and yeast (An et al., 2015). Likely, *L. sakei* GM3 displayed a broad spectrum of antagonistic activity against *Candida* spp., Gram-negative and positive bacterial strains (Avaiyarasi et al., 2016). Likewise, several reports detailing the antagonistic property of bacteriocin-producing microbes, and their activities were mostly varied (Ayed et al., 2015; Biscola et al., 2013; Cavicchioli et al., 2017; Gao et al., 2010).

Bacteriocin and BLIS are peptides or proteins in nature. It has been reported that the screening of the presence of BLIS in CFS required some enzymes and catalase treatment study (Ahmadova et al., 2013). Thus, we have treated the CFS with catalase, to remove H_2O_2 from the solution, and with proteinase K, to inactivate the bacteriocins and/or BLIS. Then the samples were tested against representative indicator strains. The obtained results revealed that CFS contains BLIS (Table 2). Ahmadova et al. (Ahmadova et al., 2013) reported that CFS of *E. faecium* AQ71 showed no activity against indicator strains

after treating with pronase E, protease type VIII, and protease type X, while catalase, lipase type VII, and α-amylase did not affect the activity. The results of the current study revealed that the antimicrobial activity of CFS was attributed to the presence of BLIS.

The morphological and physiological characters such as Gram-positive, cocci, catalase negative, and can grow at 45 °C in the presence of 6.5% of sodium chloride at pH 9.6 suggested that the selected LAB strains may belong to *Enterococcus* (Table S3). RAPD analysis of the strains revealed that all ten strains can be classified into three groups. The most potent strains were selected for molecular identification such as OV1-1, PKS2-1, and MP6/2, representing each clonal group, and found that OV1-1 and PKS2-1 were *E. faecium* and MP6/2 was *P. pentosaceus.* Several studies have been reported the BLIS producing *E. faecium* and *P. pentosaceus* (Ahmadova et al., 2013; Perumal & Venkatesan, 2017; Todorov & Dicks, 2009; Toit et al., 2000; Tulini et al., 2011).

The diversity of bacteriocin in *Enterococcus* spp. was described earlier (Nes et al., 2007). The bacteriocin of *E. faecium* 130, isolated from mozzarella cheese, was purified and showed antagonistic activity against Gram-positive bacteria, and activity was stable even after heat treatment at 100 °C for 15 min, and vast pH range (pH 2-10). The bacteriocin of *E. faecium* 130 was about 3.5 to 6.5 kDa (Tulini et al., 2011). Another cheese isolate and non-virulent, *E. faecium* AQ71 showed antagonistic activity against LAB, *Listeria* and *Bacillus* spp., and harboring enterocins P, A, L50A, and L50B coding genes. The bacteriocin was heat, pH, detergent, and salt stable. The esterase, acid phosphatase, esterase lipase, and aminopeptidase activities were observed in *E. faecium* AQ71 (Ahmadova et al., 2013). *E. faecalis* strains isolated from pig feces showed antibacterial activity only against *Enterococcus* spp. These bacteriocins, about 3-4 kDa in size, were heat stable and exhibited maximum activities at neutral pH. The studied seven strains of *E. faecalis* (BFE1071, BFE1072, BFE1113, BFE1170, BFE1228, BFE1229 and BFE 1263) were sensitive to α-chymotrypsin, trypsin, proteinase K, pepsin, papain, and pronase while showing resistance against lysozyme lipase, and catalase (Toit et al., 2000). Bacteriocin with broad-spectrum antibacterial activity was purified from *E. faecalis* CV7, vancomycin-resistant strain, and reveled that the bacteriocin was stable against temperature, pH, proteolytic enzymes, detergents, and solvents. It has been proved that the bacteriocin of *E. faecalis* CV7 has anti-cell proliferative property on HeLa cells. *E. faecalis* CV7 could be a probiont without any harmful genetic background (Perumal & Venkatesan, 2017). Another broad-spectrum bacteriocin producing *E. faecium* GM-1, isolated from infant feces, was described with temperature, and pH resistance, similar to enterocin P (Kang & Lee, 2005).

Pediococcus, one of the genera of Lactobacillaceae family, contains Gram-positive lactic acid bacteria, known to produce class IIa bacteriocin with commercial importance as biopreservatives (Papagianni & Anastasiadou, 2009). A bacteriocin producing *Pediococcus pentosaceus* was isolated from *Sclerocarya birrea* was reported. The bacteriocin was active against Gram-positive and Gram-negative bacteria. It had a molecular mass about 6.5 kDa and was class IIa bacteriocin. This bacteriocin was stable at pH 2-12, and temperature at 100 °C for two hours or at 121 °C for 20 min. The detergent treatments (Tween 80, Triton X-114, Tween 20, SDS, Triton X-100), urea, EDTA and NaCl treatments does not affect the activity while proteolytic enzymes inactivate. The strain *P. pentosaceus* ST44AM can produce bacteriocin at 26 °C, 30 °C and 37 °C without any changes in its activity (Todorov & Dicks, 2009). *P. pentosaceus* K34 was isolated from fermented sausages. It can produce bacteriocin named bacPPK34 with the molecular mass of 2.5-6.2 kDa. The bacPPK34 was resistant to detergents, salts, heat, and pH. The bacPPK34 was active against *L. monocytogenes* and *E. faecalis* ATCC 29212 (Abrams et al., 2011). Borges et al. (2013, 2014) reported about a bacteriocin producing *P. pentosaceus* SB83 with potential for vaginal application. This bacteriocin resists vaginal conditions and is active against *L. monocytogenes*. Jang et al. (2014) also reported a *P. pentosaceus* bacteriocin active against *L. monocytogenes.* The antimicrobial activity of *P. pentosaceus* Vtcc-B-601 against Gram-negative and -positive bacteria was reported by Nghe & Nguyen (2014). Likewise, many reports are available on bacteriocin producing *P. pentosaceus* isolated from FF like kimchi (Piva & Headon, 1994; Shin et al., 2008; Wu et al., 2004).

In the present study, CFS treated with α-chymotrypsin, proteinase and trypsin showed no activity, while catalase and heat treatment did not affect the bioactivity (Table S3 and S5). The results suggested that CFS of selected strains contains BLIS.

5 Conclusion

The data represented in the study demonstrated that the fermented products are a rich source of bioactive microbes like bacteriocin producing LAB. The studies revealed that the screened and identified strains, *P. pentosaceus* and *E. faecium* produce BLIS showing the narrow spectrum of antagonistic activities. The BLIS of *P. pentosaceus* and *E. faecium* were heat stable, and pH resistant. Further characterization of bacteriocin and the evaluation of probiotic properties and absence of virulence factors and antibiotic resistance genes of *E. faecium* and *P. pentosaceus* are under investigation. With these data confirming the absence of detrimental genes, *P. pentosaceus* and *E. faecium* could be used as a starter culture to prepare fermented products and also be used as biopreservative agents.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Primers used in this study.

Table S2. Lactic acid bacteria isolates were isolated from fermented products.

Table S3. Morphology and physiological characteristics of isolates.

Table S4. Results of RAPD fingerprinting BLIS-producing LAB isolates using three primers.

Table S5. Activity of bacteriocin in neutralized cell free supernatants after heating against Listeria ivanovii revealed by agar well diffusion assay.

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