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Preliminary Evaluation of Anti-Listerial Bacteriocin-like Peptide Produced by *Enterococcus lactis* PMD74 Isolated from Ezine Cheese

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ABSTRACT

Enterococcus lactis PMD74 is a novel strain with a notably high antimicrobial activity. The present study evaluated the anti-listerial effect of a bacteriocin-like peptide (BLIP) produced by *E. lactis* PMD74 isolated from Ezine cheese (PDO). The strain was screened for its antimicrobial activity against 22 indicator strains using both agar spot and well diffusion methods. We observed that the neutralized cell-free supernatant (CFS) of *E. lactis* PMD74 exhibited varying levels of antimicrobial activity against both closely and distantly related pathogenic strains, with the highest activity displayed against *Listeria monocytogenes* strains. Although thermostable and resistant to lysozyme treatment, BLIP could be completely inactivated by trypsin, proteinase K, and α-chymotrypsin treatments. BLIP production starts in the early exponential growth phase of *E. lactis* PMD74 (3 h incubation, 400 AU mL^{-1}) and reaches its maximal production (6400 AU mL^{-1}) at the end of the exponential growth phase. Moreover, it is stable in the pH range of 2.0 to 7.0. The treatment of cultures of *L. monocytogenes* ATCC 7644 and *Escherichia coli* ATCC 26922 with sterilized CFS exhibited bactericidal and bacteriostatic effects, respectively. Furthermore, co-inoculation of *L. monocytogenes* ATCC 7644 and *E. lactis* PMD74 in skim milk led to complete loss of viability of *L. monocytogenes* ATCC 7644. These findings suggest that BLIP produced by *E. lactis* PMD74 could serve as a promising food preservative agent owing to its bactericidal and bacteriostatic properties.

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Introduction

Listeria monocytogenes is a ubiquitous pathogen, the causative agent of listeriosis, which is associated with high mortality, especially in the immunocompromised individuals such as newborns, elderly, and pregnant women (Al-Nabulsi et al., 2015; Şanlıbaba et al., 2018a). According to the 2013 and 2016 reports published by the European Food Safety Authority (EFSA), increasing incidences of listeriosis have been found in the member states (EFSA 2017; Ricci et al., 2018). Although the data from South Africa lack regular documentation, 18.71% of the patients infected with listeriosis have been reported to die in March 2018 (NICD, 2018). *Listeria* spp. could be isolated from several different environments, including foods. The ready-to-eat (RTE) products that are consumed without prior cooking are very prone to contamination with *L. monocytogenes* and act as a reservoir for this pathogen. Despite the implementation of strict food safety standards, the European Union (EU) and United States (US) have adopted a zero-tolerance policy against *L. monocytogenes* in RTE food products (Neria et al., 2019). Being a psychrotrophic microorganism, *L. monocytogenes* can tolerate and grow in adverse environmental conditions such as refrigeration temperatures, extreme pH values of 4.39 to 9.40, high salinity (40% w/v), low water content, and hypoxic conditions (Al-Nabulsi et al., 2015), allowing it to adapt to variable conditions and making it difficult to control it in the food industry. Although contamination by *L. monocytogenes* during food processing could be avoided using different strategies, failure of hygiene practices and incorrect design of equipment are majorly responsible for its continuous presence in the environment, thereby increasing the risk of cross-contamination (Melero et al., 2019). For effective control of this species, alternative strategies to conventional preservation methods, such as chemical additives, excessive salt to control the growth and/or survival of *L. monocytogenes*, are imperative. The recent literature cites the use of purified or semi-purified bacteriocins, one of the antimicrobials produced by lactic acid bacteria (LAB) as a starter, protective, or adjunct culture, as the most intensively studied strategies (Yang et

al., 2012; Fontana et al., 2015; Silva et al., 2016; Hwanhlem et al., 2017). These bacteriocins are harmless to eukaryotic cells and are readily digested by proteolytic enzymes of the digestive system, owing to which these are considered as a good alternative to chemical preservatives (Aspri et al., 2017). Structurally, bacteriocins, synthesized ribosomally by LAB, are small, cationic, amphiphilic (rather hydrophobic) antimicrobial peptides or proteins that vary in their spectrum and mode of activity. These have different molecular structure and molecular mass, thermostability, pH range of activity, and genetic determinants (Klaenhammer, 1993; Moreno et al., 2006). Since1955 when the first bacteriocin-like substance was isolated from the group D streptococci (Kjems, 1955), a large number of enterocins have been studied to date (Moreno et al., 2006). The anti-listerial activity of bacteriocin was first described by Krämer and Brandis (1975). This phenomenon that can be explained by the close phylogenetic relationship of both genera is well documented today. The primary producers of characterized and purified enterocins are *Enterococcal faecalis* and *Enterococcal faecium* strains; these enterocins are grouped into class I, class IIa, class IIc, and class III bacteriocins (Moreno et al., 2006). Moreover, many recent studies have led to the isolation and identification of novel enterococcal strains, excluding *E. faecalis*, which are widely used in the food industry and suitable if consumed by both human and animals (Sonsa-Ard et al., 2015; Aspri et al., 2017; İspirli et al., 2017; Du et al., 2017; Braïek et al., 2018a). *E. lactis*, which was first isolated from the Russian sour milk products (Botina and Sukhodolets, 2006) and later in South Africa from fresh sheep milk (Bauer et al., 2009), was described by Morandi et al. (2012) as a novel species belonging to the genus *Enterococcus*. Braïek et al. (2018a) investigated and described in detail the potential use of bacteriocin produced by *E. lactis* strain, isolated from shrimps, as a natural agent for preserving food. Ezine cheese (PDO) contains biodiversity of spontaneous LAB microbiota and therefore constitutes a rich source for the isolation of novel strains. For example, a novel strain of *E. lactis*, as identified through molecular methods, with notably high antimicrobial activity was isolated from PDO. This strain has been deposited in the NCBI GenBank database under the accession number MK318965–66 as *E. lactis* PMD74.

The present study evaluated the anti-listerial effects of *E. lactis* PMD74 and characterized its bacteriocinogenic potential. To the best of our knowledge, this study is the first of its kind to investigate the bacteriocin-producing ability of *E. lactis* strain in our country. *L. monocytogenes* is regarded as a major risk factor for food safety; therefore, pioneer studies focusing on the isolation of anti-listerial bacteriocin-producing strains are considered to serve as the database for its control.

Materials and Methods

Bacterial Strains and Growth Conditions

E. lactis PMD74 strain was grown overnight in de Man Rogosa Sharpe (MRS; Merck, Germany) medium at 37°C. Table 1 enlists the source and growth conditions of the indicator strains that were used in the present study. All indicator strains were stored at –20°C in their corresponding suitable broth media supplemented with 20% glycerol. Working cultures for each strain were prepared by transferring the respective frozen stock culture to the appropriate broth, followed by an overnight incubation to revive the cells.

Table 1 The indicator strains and the antimicrobial activity spectrum of *E. lactis* PMD74

Strains	Source[a]	Growth conditions[b]	Antimicrobial activity[c]	
			Agar spot test	Well diffusion
Lactobacillus plantarum LMG2003	NLH	MRS, 37°C	+++	NZ
Lactobacillus sake NCDO2714	NLH	MRS, 37°C	+++	+++
Lactococcus lactis SIK83	NLH	M17, 30°C	NZ	NZ
Pediococcus pentosaceus FBB611	NLH	MRS, 37°C	+++	++
Pediococcus pentosaceus BH105	AUFF	MRS, 37°C	NZ	NZ
Bifidobacterium longum CHL28	NLH	MRS, 37°C	++	NZ
Bacillus cereus FM1	NLH	LB, 37°C	NZ	NZ
Listeria monocytogenes ATCC 7644	AUFF	TSB-YE, 35°C	+++	+++
Listeria monocytogenes L35	AUDFE	TSB-YE, 35°C	+++	++
Listeria monocytogenes L38	AUDFE	TSB-YE, 35°C	+++	++
Listeria monocytogenes L39	AUDFE	TSB-YE, 35°C	+++	+
Listeria monocytogenes L47	AUDFE	TSB-YE, 35°C	+++	+++
Listeria monocytogenes L48	AUDFE	TSB-YE, 35°C	+++	+
Listeria monocytogenes L49	AUDFE	TSB-YE, 35°C	+++	++
Listeria monocytogenes L74	AUDFE	TSB-YE, 35°C	+++	+++
Staphylococcus aureus ATCC 6538	AUFF	LB, 37°C	+++	++
Salmonella enterica serotype Typhimurium SL1344	AUFF	LB, 37°C	+++	NZ
Micrococcus luteus NCIMB8166	AUFF	LB, 37°C	NZ	NZ
Enterococcus faecalis LMG2708	NLH	BHI, 37°C	+++	++
Enterococcus faecalis ATCC 29212	AUFF	BHI, 37°C	+++	++
Escherichia coli ATCC 26922	AUFF	LB, 37°C	+++	++
Escherichia coli LMG3083 ETEC	NLH	LB, 37°C	+++	++

[a]NLH: Agricultural University of Norway, AUFF: Faculty of Science, University of Ankara, Turkey, AUDFE: Department of Food Engineering, University of Ankara, Turkey, [b]MRS: de Man Rogosa Sharpe (Merck, Germany), BHI: Brain Heart Infusion (Merck, Germany), LB: Luria Bertani (Merck, Germany), M17 (Merck, Germany), [c]NZ: No inhibition zone, diameter of inhibition zones +: 1–5 mm, ++: 6–10 mm, +++: ≥11 mm

Evaluation of Antimicrobial Activity

The *E. lactis* strain was screened for its antimicrobial activity spectrum using both agar spot test (van Belkum et al., 1989) and well diffusion test (Tagg and Mcgiven, 1971) against the members of the genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Bifidobacterium*, *Listeria*, *Micrococcus*, *Bacillus*, *Staphylococcus*, *Salmonella*, and *Escherichia* (Table 1).

Approximately, 10^5 to 10^6 CFU mL^{-1} of indicator strains were inoculated into BHI, MRS, M17, and LB soft agar and poured over the MRS plates that were pre-seeded with *E. lactis* PMD74.

To perform the well diffusion assay, the cell-free supernatant (CFS) was collected from an overnight culture of the test strain by centrifugation (10000 ×g, 15 min at 4°C). The pH of the CFS was adjusted to 6.5 using 6 mmol L^{-1} NaOH, and sterilized using a 0.45-µm pore size membrane filter (Millipore, France). The sterilized CFS (100 µL) was placed into wells made into the medium containing the indicator strains. After 24 h of incubation at the optimal growth temperature of the respective indicator strains, the plates were examined for inhibition zones. The diameters (mm) of inhibition zones were scored as: NZ (no inhibition zone), + (a clear zone of 1–5 mm), ++ (a clear zone of 6–10 mm), and +++ (a clear zone of ≥ 11 mm). The proteinous structure of the CFS was determined by its initial degradation by proteolytic enzymes proteinase-K (Thermo Fischer Scientific, United States), trypsin (Sigma Aldrich, Germany), and catalase (Sigma Aldrich, Germany), at a final concentration of 1 mg mL^{-1}, against the indicator strain *L. monocytogenes* ATCC 7644. The activity was expressed in arbitrary unit per milliliter (AU mL^{-1}), with one AU defined as the highest dilution exhibiting a clear zone of inhibition (Daba et al., 1993). The experiment was performed in triplicate.

Partial Characterization of Bacteriocin-Like Peptide

The effects of enzymes, pH, and heat treatments on the activity and stability of BLIP served as partial characterization (Uymaz et al., 2009). Briefly, the CFS of an overnight culture was obtained as described earlier.

In order to test the effect of enzymes on the activity of BLIP, the CFS was treated with the following enzymes at a final concentration of 1 mg/mL: trypsin (pH 7.0; Sigma Aldrich, Germany), α-chymotrypsin (pH 7.0; Sigma Aldrich), proteinase K (pH 7.0; Thermo Fischer Scientific, United States), pepsin (pH 7.0; Sigma Aldrich), α-amylase (pH 7.0; Sigma Aldrich), lipase (pH 7.0; Sigma Aldrich), catalase (pH 7.0; Sigma Aldrich), and lysozyme (pH 7.0; Sigma Aldrich). The reaction mixtures were incubated at 37°C for 2 h, following which the reactions were terminated by heating at 100°C for 5 min. The reaction mixtures and untreated supernatant, which was used as a control, were transferred to the wells in the MRS media plates.

In order to determine the effects of pH on the antimicrobial peptide of the isolate, the pH of the CSF was adjusted between 2.0 and 11.0 using 6 mmol L^{-1} NaOH or HCl. After incubation at 37°C for 2 h, the residual activity was tested. The effect of heat on the antimicrobial activity of the peptide was evaluated by heating the CFS for 5, 10, and 15 min at 80, 90, 100°C, respectively, and at 121°C for 15 min. In all treatments, the remaining activity was measured by the critical dilution method proposed by Daba et al. (1993) against the indicator strain *L. monocytogenes* ATCC 7644. The mean values were calculated from three parallel experiments.

Kinetics of Bacteriocin-Like Peptide Production

The time course of BLIP production was determined according to the method proposed by Ahmadova et al. (2013).

For this purpose, MRS broth was inoculated with an overnight culture (2% v/v) and incubated at 37°C. At regular intervals of 1 h for a total of 24 h, the optical density (OD) at 600 nm, Shimadzu, Japan) of the culture and changes in pH (Sartorius, United States) were determined. The antimicrobial activity of the BLIP in the CFS was also calculated as AU mL^{-1} as previously described, every hour for a total of 24 h.

Inhibition of Pathogen

The effects of BLIP on the growth of pathogens were evaluated both spectrophotometrically (Ahmadova et al., 2013) and through viable cell count in the fermented milk co-inoculated with the pathogen and the isolate (Favaro et al., 2014).

Briefly, the CFS of *E. lactis* PMD74 was prepared as described earlier. *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 26922 were used as Gram-positive and Gram-negative pathogens, respectively. Filter-sterilized CFS (10%) was added to the cultures of indicator strains during the early exponential phase (3-h-old culture) and incubated at 37°C. The optical density at 600 nm was measured at 1 h intervals for a total of 12 h.

The overnight cultures of *L. monocytogenes* ATCC 7644 and *E. lactis* PMD74 were co-inoculated (2% from each culture, v/v) into skimmed milk (4%, w/v) that had been previously sterilized and cooled to room temperature. The inoculated milk was then incubated at 37°C for 24 h to allow fermentation, followed by storage at 4°C for 4 weeks. *L. monocytogenes* ATCC 7644 (2%, v/v) skimmed milk was used as control. During storage, the co-inoculated fermented milk and control were examined every week for the viability of each bacterium (expressed as Log CFU mL^{-1}) and changes in pH.

Statistical Analysis

All statistical analyses were performed using the SPSS program (version 17; SPSS Inc., Chicago, IL, United States). Means and standard deviations were calculated and the significant differences were determined using analysis of variance (ANOVA) at the probability level of $P<0.05$.

Results

In the present study, we screened the antimicrobial activity spectra of the strain *E. lactis* PMD74 against different pathogenic and non-pathogenic 22 indicator strains using both agar spot and well diffusion methods. The agar spot assay helped in determining the broad antimicrobial activity spectrum, including the Gram-negative bacteria, as presented in Table 1 ($P<0.05$). We observed that the neutralized CFS of *E. lactis* PMD74 exhibited antimicrobial activity against both closely related and unrelated Gram-positive bacteria. The treatment of neutralized CFS isolated from *E. lactis* PMD74 with

proteinase-K and trypsin resulted in a complete loss of its antimicrobial activity against *E. faecalis* ATCC 29212. However, no alteration in the antimicrobial activity was observed with catalase treatment (data not shown). The antimicrobial activity of BLIP was calculated to be 6400 AU mL^{-1}.

The activity of BLIP was completely inactivated by trypsin, proteinase K, and α-chymotrypsin treatments. Although the BLIP peptide displayed resistance to lysozyme treatment, we observed a 50% reduction in its antimicrobial activity after treatment with other enzymes. The activity was unaffected by heat treatment at 80°C for 5 and 10 min; however, it decreased to 50% after treatment at 90 and 100°C. Interestingly, heat treatment at 121°C for 15 min could not completely inactivate the peptide. With respect to the effect of pH, the antimicrobial activity of BLIP was found to remain stable at pH 2.0 to 7.0; however, increasing the pH level to 8.0 led to the variable percentage of activity loss ($P<0.05$) (Table 2).

Table 2 Effect of enzymes, temperature, and pH treatment on bacteriocin activity

Application	Activity (AU mL^{-1}) *E. lactis PMD74*
Control	6400
Enzymes	
Trypsin	-
α-chymotrypsin	-
Proteinase K	-
Pepsin	3200
α-amylase	3200
Lipase	3200
Catalase	3200
Lysozyme	6400
Temperature	
80°C/5 min	6400
80°C/10 min	6400
80°C/15 min	3200
90°C/5 min	3200
90°C/10 min	3200
90°C/15 min	3200
100°C/5 min	3200
100°C/10 min	3200
100°C/15 min	1600
121°C/15 min	800
pH	
2	6400
3	6400
4	6400
5	6400
6	6400
7	6400
8	3200
9	3200
10	1600
11	1600

Figure 1 shows the kinetics of BLIP production and growth of *E. lactis* PMD74. It was observed that the BLIP production started in the early exponential growth phase (3 h incubation, 400 AU mL^{-1}) and reached its maximum production (6400 AU mL^{-1}) at the end of this phase (8 h of incubation). During the stationary growth phase of the strain, 50% to 75% decrease in the production of BLIP was recorded. According to these results, *E. lactis* PMD74 strain was determined as a low acidifier owing to the pH of the earlier phase of growth (5 h incubation) to be below 5.0, after which there was negligible change in the pH during next 24 h of incubation (data not shown).

Figure 2a and 2b summarize the inhibitory effects of BLIP produced by *E. lactis* PMD74 on pathogens. The treatment of cell cultures of *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 26922 with sterilized CFS in the early exponential growth phase (3 h) inhibited their growth, which is attributed to the maximum production of BLIP during this phase. The observed decrease in the OD immediately after the addition of CFS (Figure 2a) could be explained by the lysis of cells of *L. monocytogenes* ATCC 7644. On the other hand, cell lysis was recorded after 8 h of treatment in the case of *E. coli* ATCC 26922. Co-inoculation of skim milk with *L. monocytogenes* ATCC 7644 and *E. lactis* PMD74 resulted in complete loss of viability of *L. monocytogenes* ATCC 7644 after fermentation. However, the controlled growth of *L. monocytogenes* ATCC 7644 did not affect the viability during the storage period. On the other hand, viable counts of the strain *E. lactis* PMD74 remained almost consistent (Figure 3).

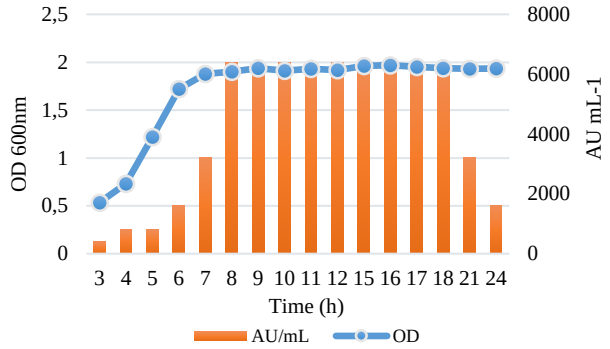

Figure 1 Time course of bacteriocin-like peptide production by *E. lactis* PMD74

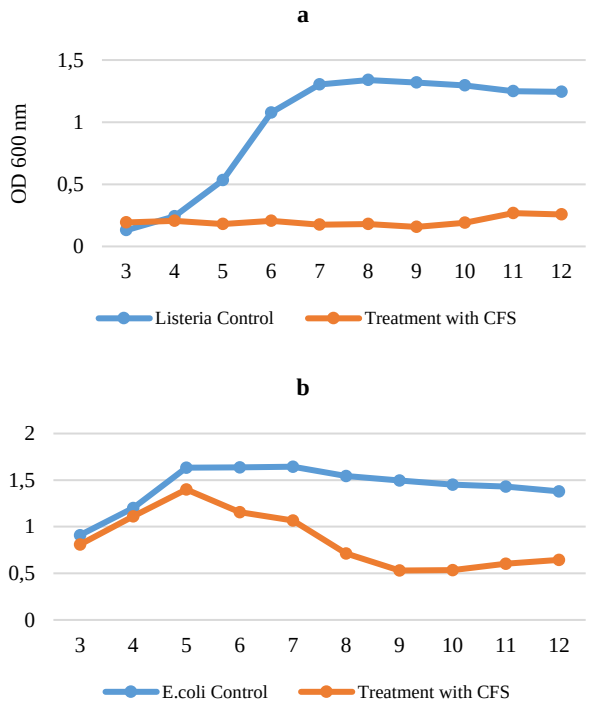

Figure 2 Effect of CFS of *E. lactis* PMD74 on the growth of *L. monocytogenes* ATCC 7644 (a) and *Escherichia coli* ATCC 26922 (b)

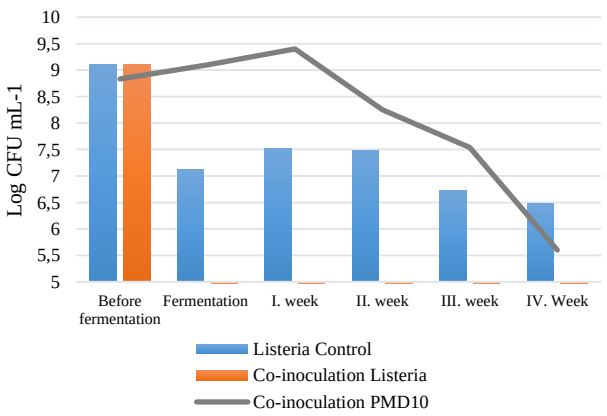

Figure 3 The viability of *L. monocytogenes* ATCC 7644 and *E. lactis* PMD74 in co-inoculated skim milk during storage

Discussion

The results of the present study report *E. lactis* PMD74-mediated inhibition of growth of closely related Gram-positive bacteria and Gram-negative pathogenic strains to varying levels. This inhibitory activity is associated with the production of the bacteriocin like peptide BLIP by *E. lactis* PMD74. Similar spectra of inhibitory activity by different enterococcal strains have been reported previously by other authors as well (Ahmadova et al., 2013; İspirli et al., 2017; Braïek et al., 2018a), suggesting that the present study was noteworthy and significant in documenting the antimicrobial activity of *E. lactis* PMD74 against all tested *L. monocytogenes* strains. The highest activity of the neutralized CFS was detected against *L. monocytogenes* ATCC 7644, *L. monocytogenes* 47, and *L. monocytogenes* 74. Listeriosis, caused by *L. monocytogenes*, is one of the most important bacterial infections worldwide, with several studies reporting its increasing occurrence (EFSA 2017, Ricci et al., 2018). Although studies on the antimicrobial activity of *E. lactis* PMD74 are sparse in our country, Şanlıbaba and colleagues demonstrated the high prevalence of *L. monocytogenes* in RTE foods (2018a) and dairy products (2018b, 2018c). To determine the anti-listerial activity of *E. lactis* PMD74, *L. monocytogenes* strains isolated from RTE foods were selected as indicator strains. The results of the present study were observed to be consistent with those reported in the study by Braïek et al. (2018b; 2018c), who described the antimicrobial effects of *E. lactis* against *Enterococcus* spp. and *Listeria* spp. The observed high anti-listerial effect of the tested strain is in line with the studies reported previously by two different groups (Belgacem et al., 2010; Ahmadova et al., 2013). However, the activity of *E. lactis* PMD74 against *L. monocytogenes* in the present study was reported to be considerably higher than that reported in the study conducted by Braïek et al. (2018b; 2018c). The loss of antimicrobial activity of the neutralized CFS upon treatment with proteinase-K and trypsin indicated the proteinaceous nature of the antimicrobial compound. The antimicrobial activity of *E. lactis* PMD74, which was found to be higher than that reported for *E. faecium* by Sonsa-Ard et al. (2015) and Ahmadova et al. (2013) (1828 AU mL^{-1} and 3200 AU mL^{-1}, respectively), was estimated to be 6400 AU mL^{-1}. This is more than that estimated by Braïek et al. (2018a) who recorded the maximum activity for *E. lactis* strains to be 1400 AU mL^{-1}.

Although treatment of CFS with proteolytic enzymes led to a total loss of its activity, treatment with other enzymes such as catalase, lipase, lysozyme, and α-amylase did not have any effect. Insensitivity to catalase could be explained by the fact that the antimicrobial activity of *E. lactis* PMD74 is not related to the production of H_2O_2 (Ahmadova, 2013). The inability of lipase and lysozyme to degrade BLIP is related to the absence of a requirement of a lipid moiety in the peptide. Retention of antimicrobial activity upon treatment with lipase and lysozyme also indicated BLIP to be pure protein molecule rather than a conjugated one (Osmanağaoğlu et al., 2007; Rivas et al., 2012). Heating the CFS at 90 and 100°C has been reported to cause a 50% decrease in the activity. Regardless of the antimicrobial decrease observed after heating at 121°C, the

BLIP was regarded as heat stable since its activity was not completely lost. Thermostability has been reported to be a common property of bacteriocins produced by *Enterococcus* spp. Moreover, it is a crucial characteristic of a bacteriocin to be used as a food preservative, since many food-processing procedures involve a heating step (Aspri et al., 2017). The thermostability displayed by the enterocin-like substance tested was compatible with the results obtained from studies on other bacteriocinogenic *E. faecium* strains (Belgacem et al., 2010; Rivas et al., 2012; Ahmadova et al., 2013). Furthermore, the observed wide pH range stability of BLIP produced by *E. lactis* PMD74 was similar to that reported in the previous studies on bacteriocins of *E. faecium* (Ahmadova et al., 2013; Favaro et al., 2014; Aspri et al., 2017). pH stability is another trait that enhances the potential of bacteriocin as a natural preservative for foods.

The BLIP could be regarded as a secondary metabolite owing to its highest production in the in the stationary phase. This finding is compatible with that reported by a previous study on bacteriocins produced by *E. faecium* (Favaro et al., 2014).

The BLIP produced by *E. lactis* PMD74 was shown to exert certain remarkable effects on the growth of pathogens. The results of the present study indicated the inhibition of cell growth to be dependent on the indicator strain used. For instance, BLIP caused lysis of cells of *L. monocytogenes* ATCC 7644, which could be explained by its bactericidal effect; however, it exhibited a bacteriostatic action on the *E. coli* strain ATCC 26922. These results are in line with those reported by Ahmadova et al. (2013). The ability to survive and inhibit the pathogen in the acidic conditions of fermented milk for four weeks is also suggestive of its potential use in food systems as a bio-preservative.

The genus *Enterococcus* is usually not considered to be a "generally recognized as safe" (GRAS). Enterococci are also known to be opportunistic pathogens and are associated with developing nosocomial infections, indicating their role as a food preservative to be controversial (Franz et al., 2011; Bigwood et al., 2012). However, virulence factors responsible for the pathogenicity are strain specific, with their expression depending on several environmental factors (Carlos et al., 2010). Therefore, the potential of some of these strains to exert beneficial effects always exist.

Conclusion

The results of the present work indicate that BLIP produced by the newly isolated strain *E. lactis* PMD74 could serve as a potential bio-preservative against food-borne pathogens to increase food safety. The remarkably high inhibitory activity of BLIP recorded against *Listeria* spp. will be a pioneer finding for future studies on the control of *L. monocytogenes* that has emerged as a major health concern worldwide, especially in foods. The present study could be considered as a preliminary study to identify BLIP as an enterocin produced by *E. lactis* PMD74. To conclude, with respect to food safety, more studies are warranted to determine the structural genes coding for the known enterocins. The strain also should be evaluated respect of safety.

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