Annals of Microbiology, **59 (3)** 485-491 (2009)

In vitro characterization of probiotic properties of *Pediococcus pentosaceus* BH105 isolated from human faeces

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Received 30 March 2009 / Accepted 15 June 2009

Abstract - In this study, a new bacteriocin-producing strain *Pediococcus pentosaceus* BH105 was isolated from human faeces and subsequently *in vitro* probiotic and antagonistic properties were investigated. This strain exhibited high viability at pH 3.0 and in the presence of pepsin, pancreatin and bile salts (0.3%). BH105 was sensitive to 19 of 25 antibiotics and showed no haemolytic activity. The bacteriocin produced by BH105 was active at wide pH range of 2.0 to 11.0 and was heat stable at 80, 90 and 100 °C for 15 min. Bacteriocin activity was inhibited by pepsin, α-chymotrypsin, and proteinase K but not by trypsin, α-amylase, catalase, lysozyme and lipase. The Tricine-SDS-PAGE analysis allowed an approximation of bacteriocin BH105 size about 5 kDa. *Pediococcus pentosaceus* BH105 was able to adhere to Caco-2 cells (10.12 ± 2.40%) and inhibited the adhesion of *Escherichia coli* LMG3083 (88.72 ± 5.53%) and *Salmonella typhimurium* SL1344 (60.64 ± 10.97%).

Key words: *Pediococcus pentosaceus*; probiotic; bacteriocin; adhesion.

INTRODUCTION

Probiotics are defined as "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Mottet and Michetti, 2005). Probiotic microorganisms include many strains of lactic acid bacteria (LAB), and selected strains have activity on intestinal microbiota. It is well known that probiotics have to transiently colonize to the human gut. Best effect is achieved if the microorganisms colonise the intestinal surface mucus layer since they then can affect the intestinal immune system, displace enteric pathogens, provide antioxidants and antimutagens, and possibly other effects by cell signalling (Vinderola *et al.,* 2004; Mottet and Michetti, 2005).

The normal intestinal microflora participates in the barrier effect by developing antimicrobial activity against enteropathogen bacteria. Bacteriocins produced by predominant LAB strains play an important role in contributing to this antimicrobial activity as well as other metabolites. Thus, bacteriocin-producing bacteria able to inhibit growth or to exclude enteric pathogens in the intestinal milieu have distinct advantages (Oh *et al*., 2000). Considerable numbers of bacteriocins have been isolated and identified from LAB including nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins, pediocins and plantaricins (Savadogo *et al*., 2006). However, a few of bacteriocin-producer LAB strains have been extensively studied for their potential use as probiotics (Oh *et al*., 2000; Savadogo *et al*., 2006). There is an increasing interest in isolating new bacteriocin-producing strains of human intestinal origin that could be employed for probiotic effects and inhibition of pathogenic bacteria in the gut (Mathys *et al*., 2007). *Pediococcus* strains are not traditionally considered to be natural inhabitants of the human gastrointestinal tract. Recently, Millette *et al*. (2007) have reported a bacteriocin-producing *Pediococcus acidilactici* MM39 isolated from human male stool. However, in that study the probiotic characteristics of *P. acidilactici* have not been evaluated. Since *Pediococcus* strains are widely used as starter bacteria in manufacturing fermented meat and vegetable products, establishing the effective probiotic properties of *Pediococcus* strains could lead to development of new probiotic foods.

The aim of this study was the *in vitro* assessment of the probiotic potential of a *Pediococcus pentosaceus* strain, isolated from faecal material of healthy human in order to select a candidate probiotic strain for further investigation. In this point of view probiotic characteristics of the strain such as acid and bile tolerance, adherence to Caco-2 cells and the antagonistic effect against certain bacterial strains, are described.

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MATERIALS AND METHODS

Bacterial strains and media. Table 1 lists source and growth media of bacterial indicator strains. Bacterial stocks were stored at -80 °C in their respective broths supplemented with 30% glycerol.

Isolation and identification of the strain *Pediococcus pentosaceus* BH105. The strain was isolated from faecal specimen of a healthy volunteer. Faecal sample was homogenized in 0.8% NaCl and subsequently plated on MRS agar. Following the anaerobic incubation at 37 °C for 48 h, MRS soft agar (0.7%) inoculated with 1% indicator strain (*Lactobacillus sake* NCDO2714) was overlaid on to the colonies. After 24 h of incubation at 37 °C, colonies having antimicrobial activity were selected according to their inhibition zones against indicator strain, which were larger than 5 mm. These colonies were cultivated in MRS broth and kept for further examinations.

Gram staining, motility, growth at different temperatures, CO_2 production from 0.5% glucose in MRS, arginine hydrolysis and catalase tests were used in the preliminary characterization of the isolates. API50CHL test kits were used for biochemical identification of the isolates (bioMerieux, Inc., France). The identification of isolates was performed by 16S rDNA sequence analysis. Genomic DNA was isolated with using the genomic DNA isolation and purification kit (Fermentas, Finland) and the region from nucleotide 8 to 928 of 16S ribosomal gene was amplified by PCR. Techne-TC.512 Thermocycler (England) with the universal primers 16S forward (5′-CCG TCA ATT CCT TTG AGT TT-3′) and 16S reverse (3′-AGA GTT TGA TCC TGG CTC AG-5′) (Beasely and Saris, 2005) were employed. Amplified PCR fragments were purified by the PCR purification kit (Qiagen, USA) and were sequenced by REFGEN Biotechnology (METU Technocity, Ankara, Turkey). Basic local alignment search tool (BLAST) was used to compare the sequences against the nucleotide database in National Centre for Biotechnology Information (NCBI) (http://www. ncbi.nlm.nih.gov.tr/BLAST).

Survival under conditions simulating the human GI tract. For acid tolerance assay, *P. pentosaceus* BH105 was incubated in MRS broth at 37 °C for 18 h, and 1 mL of culture was harvested by centrifugation (10000 *x g*, 5 min, 4 °C). Cells were washed three times with PBS at pH 7.2 and then resuspended in 9 mL of PBS solution adjusted to pH 1.0 and 3.0 with 5 M HCl, respectively. The viability was determined at 0, 1 and 3 h of incubation, on MRS agar plate (Conway *et al*., 1987; Maragkoudakis *et al*., 2006).

The methods described by Convay *et al*. (1987) were used to determine the tolerance of *P. pentosaceus* BH105 against pepsin (3 mg mL^{-1}; Merck) at pH 2.0 and 3.0, pancreatin (1 mg mL^{-1}, Fluka) at pH 8.0 and bile salt (0.3 % Ox-Bile, Meck).

Antibiotic resistance and haemolytic activity. The antibiotic susceptibility of *P. pentosaceus* BH105 was evaluated on MRS agar plates inoculated with 10^6 CFU mL^{-1} (OD_{600} = 0.08-0.1) of BH105 cells by using the antibiotic discs reported in Table 2. The diameters of inhibition zones were measured after incubation at 37 °C for 18 h. (Charteris *et al*., 1998).
For testing haemolytic activity, strain BH105 was plated on Columbia agar containing 5% (w/v) human blood and incubated at 30 °C for 48 h. Blood agar plates were examined for β-haemolytic (clear zones), α-haemolytic (green-hued zones) or γ-haemolytic (no zones) activity (Maragkoudakis *et al*., 2006).

Antimicrobial activity and characterization of the inhibitory compound. The antimicrobial activity of bacterial isolates was tested by both agar overlay and well diffusion assays (Van Belkum *et al*., 1989).

TABLE 1 - The antimicrobial activity spectrum of *Pediococcus pentosaceus* BH105

Strains	Source[a]	Media[b]	Anticrobial activity[c]	
			Agar overlay	Well diffusion
Lactobacillus plantarum LMG2003	NLH	MRS	+++	+++
Lactobacillus sake NCDO2714	NLH	MRS	+++	+++
Lactococcus lactis SIK83	NLH	M17	++	NZ
Lactococcus lactis IL1403	NLH	M17	++	NZ
Lactococcus lactis JC17	NLH	M17	+	++
Pediococcus pentosaceus FBB611	NLH	MRS	+++	++
Bacillus subtilis ATCC6633	AUFF	LB	+++	NZ
Bacillus cereus FM1	NLH	LB	++	NZ
Listeria monocytogenes ATCC 15813	AUFF	LB	+++	++
Listeria monocytogenes ATCC 25295	AUFF	LB	++	++
Listeria innocua LMG 2813	NLH	LB	++	++
Staphylococcus aureus ATCC 6538	AUFF	LB	+++	NZ
Stapylococcus aureus FRI 100	NLH	LB	+++	NZ
Micrococcus luteus NCIMB8166	AUFF	LB	++	NZ
Enterococcus faecalis LMG2708	NLH	MRS	+++	++
Escherichia coli LMG3083 ETEC	NLH	LB	+++	NZ
Escherichia coli ATCC 25295	AUFF	LB	+++	NZ
Salmonella enterica serotype Typhimurium SL1344	AUFF	LB	+++	NZ
Salmonella enterica serotype Typhimurium LT2	AUFF	LB	+++	NZ
Salmonella enteridis DMC3	AUFF	LB	+++	NZ

[a] NLH: Agricultural University of Norway, AUFF: Faculty of Science, University of Ankara, Turkey.
[b] MRS: de Man-Rogosa-Sharpe, LB: Luria-Bertani.
[c] NZ: no inhibition zone, +: 1-5 mm, ++: 6-10 mm, +++: 11 mm, and over diameter of inhibition zones.

To determine the effect of pH on bacteriocin activity, cell free supernatants (CFS) of the isolate *P. pentosaceus* BH105 was adjusted to pH values of 2.0 to 11.0 by using 6 N NaOH or HCl. To evaluate the effect of heat on bacteriocin activity, CFS were heated for 5, 10, 15 min at 80, 90 and 100 °C and for 15 min at 121 ºC. CFS were treated with the following enzymes at a final concentration of 1 mg/mL trypsin (pH 7.0, Merck, Germany), α-chymotrypsin (pH 7, type II, Sigma, USA), proteinase K (pH 7.0, Sigma, USA), lipase (pH 7.0, Sigma, USA), α-amylase (pH 7.0, Sigma, USA) and lyzozyme (pH 7.0, Sigma, USA). After incubation at 37 °C for 2 h, enzyme activities were stopped by heating at 100 °C for 5 min, untreated sample was used as control. The remaining bacteriocin activity was measured by critical dilution method proposed by Daba *et al*. (1993).

The inhibitory compound produced by *P. pentosaceus* BH105 was partially purified with four rounds of 40% ammonium sulphate precipitation (Sambrook *et al*., 1989). Tricine-sodium dodecyl sulphate-polyacrilamide gel electrophoresis (Tricine SDS-PAGE) was carried out for the estimation of the molecular mass of partially purified bacteriocin. Polyacrylamide concentrations in the stacking gel and separating gel were 4 and 16%, respectively. Electrophoresis was conducted at a constant voltage of 30 V for at least 1 h followed 90 V at least 12 h. After extensive 5 h of washing performed by sterile ultrapure water regularly replaced, the gel was transferred to a MRS-agar plate and overlaid with soft agar (0.7%) inoculated with a culture (OD 600 of 0.45) of the indicator strain *Lactobacillus sake* NCDO2714.

Caco-2 adhesion assay and inhibition of pathogen adhesion. The adhesion ability of *P. pentosaceus* BH105 to Caco-2 monolayer cells and its competition towards *Escherichia coli* LMG3083 (ETEC) and *Salmonella typhimurium* SLI344 for adhesion was also examined by the methods described by Maragkoudakis *et al*. (2006).

For competition assay, overnight *E. coli* LMG3083 (ETEC) and *S. typhimurium* SL1344 strains were harvested and washed with PBS buffer and resuspended in non-supplemented DMEM to achieve a population of 10^8 CFU mL^{-1}. Caco-2 cells were first challenged with *Pediococcus* strain as describe before, and washed four times with PBS. Subsequently, 1 mL of *E. coli* LMG3083 (ETEC) and *S. typhimurium* SL1344 DMEM suspension was transferred onto the Caco-2 monolayers and incubated at 37 °C in a 10% CO_2/90% air atmosphere for 90 min. Adhered bacterial cells were detached with 0.1% Tween 80 and *E. coli* LMG3083 (ETEC) and *S. typhimurium* SL1344 strains were enumerated after 18 h on McConkey agar (Maragkoudakis *et al*., 2006).

RESULTS AND DISCUSSION

Isolation and identification of *Pediococcus pentosaceus* BH105

Eighty-four different colonies giving inhibition zones larger than 5 mm against indicator strain *Lactobacillus sake* NCDO2714 on MRS agar were isolated from the human faeces samples. Among the 84 isolates, BH105 was selected due to its high antimicrobial activity and characterized as coccus, catalase negative, arginine positive growing at 15 °C but not at 45 °C. Further, API50 carbohydrate fermentation tests and 16S rDNA PCR assays showed that this strain has 99% homology with *Pediococcus pentosaceus* strain YTX21BMX (Genbank accession number FJ539347.1).

TABLE 2 - Antibiotic susceptibility of *Pediococcus pentosaceus* BH105 and *Staphylococcus aureus* ATCC 6538

Group number[a]	Antibiotic	Concentration (μg/mL)	Strains[b]	
			P. pentosaceus BH105	*S. aureus* ATCC6538
I	Penicillin G	10	S	R
	Ampicillin	10	S	R
	Ampicillin/Sulbactam	10/10	S	R
	Methicillin	5	R	R
	Cefazolin	30	R	R
	Ceftazidime	30	R	R
	Ceftriaxone	30	S	R
	Cefaclor	30	S	R
	İmipenem	10	S	R
	Meropenem	10	S	R
	Vancomycin	30	S	R
	Bacitracin	10	S	R
II	Amikacine	30	S	R
	Gentamicin	10	S	R
	Kanamycin	30	S	R
	Streptomycine	10	S	R
	Spectinomycin	100	S	R
	Tetracyclin	30	S	S
	Chloramphenicol	30	S	S
	Erythromycin	15	R	R
	Azitromycin	15	R	R
	Clindamycin	2	S	R
III	Sulfadiazine	0.25	R	R
	Trimethoprim	5	S	R
	Rifampicin	5	S	R

[a] Group I: cell wall synthesis inhibitors, Group II: protein synthesis inhibitors, Group III: nucleic acid synthesis inhibitors.
[b] Susceptibility is expressed as R: Resistance (inhibition zone < 16 mm) and S: Sensitive (inhibition zone > 16 mm) (Charteris *et al*., 1998).

Most of the probiotic strains of human origin are found to be members of the genus of *Lactobacillus* genus, including *L. acidophilus, L. plantarum, L. casei, L. rhamnosus* and *L. curvatus* (Conway *et al*., 1987; Toumola and Salminen, 1998; Maragkoudakis *et al*., 2006; Pinto *et al*., 2006). However, recently two coccus shaped strains of LAB were recovered from human adult stool, *Lactococcus lactis* MM19 and *Pediococcus acidilactici* MM33, and characterized for their bacteriocin production (Millette *et al*., 2007). Parallel to these results, *P. pentosaceus* BH105 also indicated that some members of this genus which might have probiotic potential, could survive through the intestinal tract.

Survival under conditions stimulating the GI tract

Pediococcuas pentosaceus BH105 retained its viability even after 3 h of exposure to pH 3.0. However, after 1 h at pH 1.0 complete inactivation occurred (data not shown). Although these results imply that the low pH of the stomach is a severe condition for *P. pentosaceus* BH105, it should be noted that the pH in human stomach ranges from 1.0 to 4.5 during ingestion (Conway *et al*., 1987). Therefore high resistance of the strain BH105 at pH 3.0 (100% survival) might able to accomplish the low pH of the stomach. In fact, several *Lactobacillus* strains were found to be able to retain their ability when exposed to pH values of 3.0 to 4.0, but displayed high viability loss at lower pH values (Conway *et al*., 1987; Dunne *et al*., 1999; Maragkoudakis *et al*., 2006).

Strain BH105 was also resistant to pepsin, pancreatin and bile salts (0.3%) after 1 and 4 h of treatment, respectively (Fig. 1A and 1B), but it was completely inactivated in the presence of pepsin at pH 2.0 at the end of 1 h (Fig. 1A). The resistance of BH105 to these constituents of gastrointestinal tract suggests that the relevant strain could survive in small intestine.

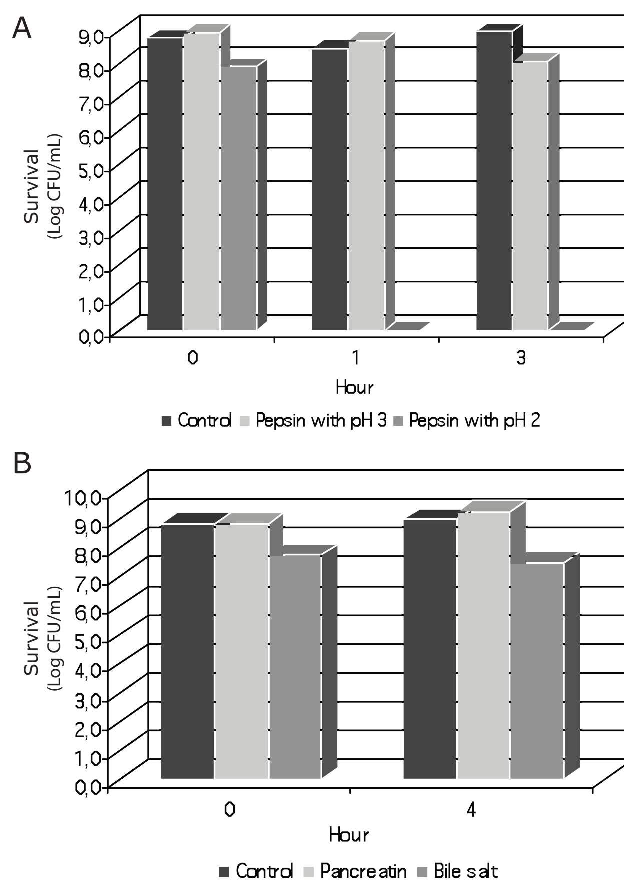

FIG. 1 - Survival of the *Pediococcus pentosaceus* BH105 strain in the presence of pepsin (A) and pancreatin and bile salt (B).

Antibiotic resistance and haemolytic activity

One of the required properties for probiotic strains is their safety for human consumption without harbouring acquired and transferrable antibiotic resistance (Pinto *et al*., 2006). The antibiotic susceptibility pattern of the strain BH105 is shown in Table 2. BH105 was found to be resistant to methicillin, cefazolin, ceftazidim, erythromycin, azitromycin and sulfadiazine among the 25 different antibiotics. These results pointed out that the tested strain has low antibiotic resistance to inhibitors of cell wall, inhibitors of protein synthesis and inhibitors to nucleic acid synthesis when compared to the other reported LAB (Zhou *et al*., 2005; Klare *et al*., 2007). In addition, BH105 showed no haemolytic activity on Colombia human blood agar plates. These results confirmed that strain BH105 could be used safely in the animal and human.

Antimicrobial activity and characterization of the bacteriocin

In the agar overlay assays, *P. pentosaceus* BH105 showed a broad inhibitory activity against all the tested indicator strains (Table 1), including Gram-negative bacteria. These results suggest that *P. pentosaceus* BH105 could have considerable antagonistic effect on enteric gram-negatives known as causes of gastroenteritis. Furthermore, neutralized CFS of BH105 showed antimicrobial activity against some related and unrelated Gram positive bacteria. In particular, the inhibitory activity against *Listeria* and *Enterococcus* (Table 1) suggests that this strain has ability to produce pediocin-like bacteriocin, like several other strains of *Pediococcus* genus such as *P. acidilactici, P. pentosaceus* and *P. parvulus* (Cintas *et al*., 1995; Albano *et al*., 2007; Anastasiadou *et al*., 2007; Millette *et al*., 2007). On the contrary, CFS of BH105 did not show any inhibitory activity against Gram negative indicator strains suggesting that the inhibitory effect is mainly due to other bacterial metabolites rather than the bacteriocin production.

Bacteriocin production in MRS broth cultures was maximal (1600 AU mL^{-1}) after 11 h, where the maximum cell density (OD_{600} 2.24) had been attained. The production of bacteriocin was related to growth curve, increasing to 1600 AU mL^{-1} during the exponential growth phase. This bacteriocin activity value remained stable throughout the stationary phase, and sharply decreased to 400 AU mL^{-1} after 24 h of fermentation (Fig. 2A). Nisin and pediocin-like bacteriocin productions occurred during the exponential growth phase but stopped when the cells reached the stationary growth phase (Cintas *et al*., 1995; Albano *et al*., 2007; Anastasiadou *et al*., 2007; Millette *et al*., 2007).

The effect of several enzymes, pH and heat treatments on the activity of the bacteriocin produced by BH105 is presented in Table 3. Protease sensitivity assay demonstrated that the antimicrobial substance produced by BH105 was a bacteriocin-like substance since its inhibitory activity was completely lost by treatment with enzyme proteinase K and α-chymotrypsin. The activity was, however, not affected by trypsin, lipase, α-amylase, catalase and lyzozyme. The bacteriocin was found to be active over a wide pH range between 2.0 and 11.0 and 50-87.5 % decreases in activity were obtained at pH 8.0-11.0. BH105 bacteriocin was completely stable under heat treatment

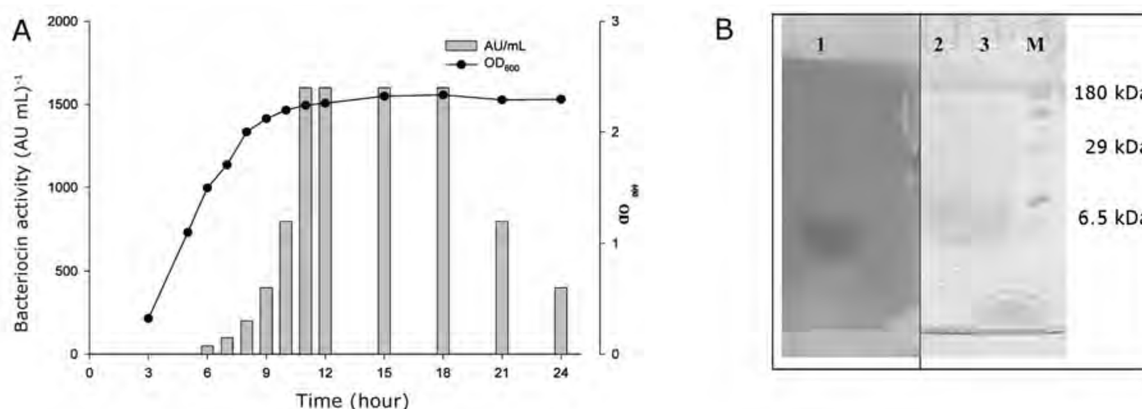

FIG. 2 - Characteristics of the bacteriocin produced *by Pediococcus pentosaceus* BH105. A) Time course of bacteriocin production and cell growth of *P. pentasaceus* BH105. B) Tricine-SDS PAGE of pediocin BH105. Lane 1: inhibition zone corresponding to the position of the peptide band in lanes 2 and 3. Lanes 2 and 3: pediocin BH105 peptide band stained with Coomassie blue. Lane M: molecular weight marker (Sigma Chem Co., USA).

at 80, 90 and 100 °C for 5, 10 and 15 min. However, activity was decreased 75% by applying sterilization temperature (121 °C for 15 min). To date pediocin-like bacteriocins were classified into two different groups for their proteolytic enzyme sensitivities which are resistant (pediocin PD-1, pediocin SA-1) or sensitive (pediocin PA-1, pediocin AcH) to both pepsin and α-chymotrypsin (Anastasiadou *et al*., 2007). Our results indicated that the antimicrobial compound present in the CFS of *P. pentosaceus* BH105 could be similar to the pediocin AcH and pediocin PA-1.

The molecular weight of the partially purified bacteriocin was analyzed by tricine SDS-PAGE. When the gel was overlaid with the indicator strain *Lactobacillus sake* NCDO2714, a single clear inhibition zone was found, corresponding to a molecular mass of about 5.0 kDa (Fig. 2B). Several authors noted that the migration of small, hydrophobic peptides in SDS-PAGE did not correlate with their true size. However the molecular weight of the small peptide shown in Fig. 2B is similar to that of most the bacteriocins reported for the genus *Pediococcus* (Anastasiadou *et al*., 2007; Millette *et al*., 2007).

Adhesion of *Pediococcus pentosaceus* BH105 to Caco-2 cells and inhibition of pathogen adhesion

Since the bacterial adhesion ability to epithelial cells has been considered as one of the selection criteria for probiotic strains, Caco-2 cell line has been used as *in vitro* model of the human intestinal epithelium to screen for adhesive strains (Conway *et al*., 1987; Toumola and Salminen, 1998; Maragkoudakis *et al*., 2006). In this study, *P. pentosaceus* BH105 showed adhesion ability (10.12 ± 2.40%), as well as control strain *E. coli* LMG3083 (10.51 ± 2.20%). Control strains *S. typhimurium* LT2 and *S. typhimurium* SL1344 displayed a lower adhesion ability than *P. pentosaceus* BH105, showing values of 5.55 ± 1.54% and 5.02 ± 1.41%, respectively. These results indicate that *P. pentosaceus* BH105 has the ability to adhere to the epithelial cells. Previous studies reported that LAB adhesive strains, such as *Lactobacillus johnsonii* La1, *Lactobacillus rhamnosus* GG, as well as *Lactobacillus casei* Shirota and *L. casei* Imunitass have an ability to adhere to Caco-2 cells ranging from 2.6 to 14.4 % (Toumola and Salminen, 1998; Ouwehand *et al*., 2001; Maragkoudakis *et al*., 2006). However, there is no information about the adhesion ability of *Pediococcus* strains.

TABLE 3 - Characterization of bacteriocin produced from *Pediococcus pentosaceus* BH105. Standard deviation is less than 5%

Treatment	Activity (AU/mL)	Treatment	Activity (AU/mL)
Control	1600	100 °C	
Trypsin (Sigma, No. T-8658)	1600	5 min	1600
α-Chymotrypsin (Sigma, No. C-7762)	-	10 min	1600
Proteinase-K (Sigma, No. P-6556)	-	15 min	1600
Pepsin (Sigma, No. P-6887)	-	121 °C, 15 min	400
α-Amylase (Sigma, No. A6380)	1600	pH	
Lipase (Sigma, No. L-1754)	1600	2	1600
Catalase (Sigma, No. C-3515)	1600	3	1600
Lysozyme (Sigma, No. L-6876)	1600	4	1600
80 °C		5	1600
5 min	1600	6	1600
10 min	1600	7	1600
15 min	1600	8	800
90 °C		9	800
5 min	1600	10	800
10 min	1600	11	200
15 min	1600		

TABLE 4 - Adhesion of bacteria on Caco-2 cells

	Escherichia coli LMG3083		*Salmonella* Typhimurium LT2		*Salmonella* Typhimurium SL1344		*Pediococcus pentosaceus* BH105	
	Live cell count (log CFU/mL)	Adhesion ability (%)	Live cell count (log CFU/mL)	Adhesion ability (%)	Live cell count (log CFU/mL)	Adhesion ability (%)	Live cell count (log CFU/mL)	Adhesion ability (%)
Replicate I	0.475 x 10^8	11.90	0.25 x 10^8	5.31	0.180 x 10^8	3.67	0.80 x 10^8	8.5
Replicate II	0.420 x 10^8	10.56	0.20 x 10^8	4.25	0.350 x 10^8	7.14	0.83 x 10^8	8.8
Replicate III	0.485 x 10^8	12.13	0.24 x 10^8	5.10	0.222 x 10^8	4.48	0.73 x 10^8	7.8
Replicate IV	0.270 x 10^8	6.74	0.32 x 10^8	6.80	0.280 x 10^8	5.71	1.21 x 10^8	12.94
Replicate V	0.450 x 10^8	11.23	0.30 x 10^8	6.30	0.200 x 10^8	4.08	1.21 x 10^8	12.94
Average	0.420 x 10^8	10.51 ± 2.20	0.262 x 10^8	5.55 ± 1.54	0.246 x 10^8	5.02 ± 1.41	0.95 x 10^8	10.12 ± 2.40

TABLE 5 - Inhibition of adhesion of *Escherichia coli* LMG3083 and *Salmonella* Typhimurium SL1344 to Caco-2 cells by *Pediococcus pentosaceus* BH105

	P. pentosaceus BH105/*E. coli* LMG3083		*P. pentosaceus* BH105/*S.* Typhimurium SL1344	
	Live cell count (log CFU/mL)	Adhesion inhibition ability (%)	Live cell count (log CFU/mL)	Adhesion inhibition ability (%)
Replicate I	0.037 x 10^8	93.98	0.053 x 10^8	57.84
Replicate II	0.049 x 10^8	91.35	0.049 x 10^8	43.62
Replicate III	0.054 x 10^8	81.20	0.037 x 10^8	65.49
Replicate IV	0.041 x 10^8	92.48	0.044 x 10^8	63.23
Replicate V	0.044 x 10^8	84.58	0.028 x 10^8	73.07
Average	0.045 x 10^8	88.72 ± 5.53	0.042 x 10^8	60.64 ± 10.97

Adhesion of *E. coli* LMG3083 and *S. typhimurium* SL1344 were reduced 88.72 ± 5.53% and 60.64 ± 10.97% respectively, when the cells were challenged with *P. pentosaceus* BH105. The inhibition ratio of pathogen adhesion to eukaryotic cell lines, has already been reported for strains *Lactobacillus johnsonii* La1, *Bifidobacterium* CA1 and F9, *Lactobacillus acidophilus* LB, *Lactobacillus paracasei* subsp. *tolerans* ACA-DC4037 and *Lactobacillus plantarum* ACA-DC to high extend (Ouwenhand *et al*., 2001; Maragkoudakis *et al*., 2006) but not as high as *P. pentosaceus* BH105. These results showed that *P. pentosaceus* BH105 could be useful in inhibition and displacement of pathogen adhesion.

CONCLUSION

Pediococcus pentosaceus BH105 is an interesting strain for its high inhibition effect on gram negative enteric pathogens as well as preventing their adhesion to epithelium cells. Additionally BH105 is an efficient bacteriocin-producer strain that resistant to hostile conditions of the digestion system. Therefore, this strain is good candidate for further investigation in vivo studies to elucidate its potential and its application as novel probiotic strain in the non-dairy fermented food industry.

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