

## Occurrence of Pediocin PA-1/AcH-Like Bacteriocin in Native Non-starter *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* from retail Cheddar cheese

Mothomang Mlalazi<sup>1</sup>, Angela R. Winslow<sup>1</sup>, Junia Jean-Gilles Beaubrun<sup>2</sup>, Broderick E. Eribo<sup>1\*</sup>

<sup>1</sup>Department of Biology, Howard University, Washington, D. C. 20059. USA

<sup>2</sup>Food and Drug Administration, Laurel, M. D. 20708.

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### Abstract

Bacteriocin producing *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus rhamnosus* were isolated from retail Cheddar cheese and identified by both phenotypic and molecular methods. These isolates were found to produce bacteriocins with broad-spectrum activity against several foodborne pathogens: *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella typhi*, *E. coli* O157:H7, *Enterococcus faecalis*, *Enterococcus faecium* and *Pseudomonas aeruginosa*. Further characterization of the bacteriocins showed that they had extremely high thermostability as activity was maintained after boiling for 6.5 h. In addition, autoclaving for 45 minutes had no significant effect on the activity. Similarly, storage at -20 °C, 4 °C, 25 °C, 37 °C and 45 °C for up to 30 days did not reduce the activity. PCR amplification analysis of the isolates with pediocin general primers identified a 332 bp DNA fragment while pediocin PA-1 specific primers identified a 1044 bp pediocin PA-1 structural gene in all the isolates. Nucleotide sequencing of the pediocin PA-1 gene showed 99% homology to *Lactobacillus plantarum* plasmid pWHE92 production operon. To the best of our knowledge, this is the first report on the presence of pediocin PA-1/AcH-like bacteriocin in other species of *Lactobacillus* outside *L. plantarum*. This study gives new insight in the occurrence of pediocin PA-1/AcH-like bacteriocin in the genus *Lactobacillus*.

**Key words:** Non-starter, *Lactobacillus*, Cheddar cheese, Pediocin PA-1, pulse field gel electrophoresis.

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### Introduction

Non-starter lactic acid bacteria (NSLAB) refers to the adventitious bacterial flora that can grow under hostile environmental conditions of cheese; 32 to 39% moisture, 4 to 6% salt in moisture, pH 4.9 to 5.3, temperature of 5 to 13°C and nutrient deficiency (Fryer and Sharpe 1996; Turner et al. 1986). The dominant NSLAB strains of Cheddar cheese are mainly mesophilic lactobacilli, although the presence of pediococci and micrococci are also known to occur. The study of NSLAB in 8 week old Irish Cheddar cheese revealed that it was composed of 55% *Lactobacillus paracasei*, 28% *Lactobacillus plantarum* and 14% *Lactobacillus curvatus* (Jordan and Cogan 1993). Several factors influence the number, extent of growth and heterogeneity of the lactobacilli. These include hygiene in the factory, the rate of cooling of the cheese following manufacture and the storage temperature (Fox et al. 1998).

The presence of adventitious NSLAB in cheese may be resulting from post-pasteurization contamination by airborne flora, the cheese-making equipment or ingredients or survival of pasteurization (Jordan and Cogan 1999; Martley and Crow 1993; Naylor and Sharpe 1958c; Reiter and Sharpe 1971).

Most studies on NSLAB of Cheddar cheese have focused on their diversity, influence on flavor development and the ultimate quality (Muehlenkamp – Ulate and Warthesen 1999; Swearingen et al. 2001, Broome et al. 1990; Beresford and William 2004). Although the role of NSLAB in Cheddar cheese is well documented, however, there have been limited studies on their bacteriocin producing potential. Such studies will be important since NSLAB with bacteriocin producing capability may confer a competitive edge against foodborne pathogens, consequently increasing the safety level of Cheddar cheese (Settani and Moschetti 2010).

Isolation and screening of microorganisms from natural processes have always been powerful tools in discovering cultures of commercial and scientific importance, therefore

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\*Corresponding author mailing address: Department of Biology, Howard University, 415 College Street, NW, Washington D.C. 20059. United States of America. Tel: +1 202 806 6937, Fax: +1 202 806 4564, Email: [beribo@howard.edu](mailto:beribo@howard.edu)

bacteriocin producing NSLAB of Cheddar cheese may not only find application in Cheddar cheese but in other dairy products. In this study, we have isolated several native non-starter Lactobacillus strains from retail Cheddar cheese showing antimicrobial activity. These isolates produce highly heat stable, and antilisterial pediocin PA-1/AcH- like bacteriocin..

## Material and Methods

**Isolation of lactic acid bacteria.** Fifteen Cheddar samples were obtained from different local supermarkets in Washington DC area. Eleven grams of each sample was aseptically transferred to a stomacher bag and mixed with 99ml of 0.01% peptone water. The content was homogenized in a stomacher laboratory blender (80, Seward, UK) for 60 s. Ten fold dilutions of the homogenates were plated on MRS agar (Remel, Lenexa, KS) and the plates were incubated anaerobically in a gas pack system at 37°C for 72 h. The viable count was then determined and isolated colonies were randomly selected based on the log number of the highest dilution on a countable plate. The selected colonies were transferred to MRS broth and purified by streaking twice on MRS agar. The pure cultures were stored at -20°C in MRS medium containing 50 % (v/v) glycerol.

**Phenotypic characterization.** Exponential phase cultures were Gram stained and screened for catalase, oxidase, and motility. The isolates with characteristic LAB profile; catalase negative, oxidase negative and non-motile, were further subjected to growth at different temperatures, salt concentration, pH, and CO<sub>2</sub> production from glucose. Biochemical analysis was performed by API 50CH system (Biomérieux, France). Identification of the isolates was done by a computerized database program provided by the manufacturer.

**Selection of LAB with antimicrobial activity.** The agar spot test method by Schillinger and Lucke (1989) was used for selection of LAB with antimicrobial activity.

**Molecular identification of isolates exhibiting antimicrobial activity.** Pure colonies were subjected to 16S rRNA sequencing (Genewiz Inc., South Plainfield, NJ). The sequences obtained were analyzed using the NCBI Nucleotide BLAST search ([www.ncbi.nlm.nih.gov/blast/html/BLASThomehelp.html](http://www.ncbi.nlm.nih.gov/blast/html/BLASThomehelp.html)).

**Preparation of genomic DNA for pulse-field gel electrophoresis (PFGE).** The bacteriocin producing LAB were cultured in MRS broth at 37°C overnight. Cells were harvested by centrifugation at 4°C for 15 min at 13,000 x g, washed once in TE buffer (10mM Tris:1mM EDTA, pH 8.0) (Sigma Aldrich St. Louis, MO) and resuspended in cell suspension buffer (100mM Tris:100mM EDTA, pH 8.0). The cell suspensions were then heated for 30 min at 65°C. After cooling for 5 min at room temperature, 50% lysozyme (Lysozyme from chicken egg white, (Sigma Aldrich, St.

Louis, MO)) was added to the cell suspensions and incubated for 30 min at 37°C. An aliquot (200 µl) of the cell suspension:lysozyme mixture was mixed with equal volume of 1.2% low melting point SeaKem Gold Agarose (Lonza, Rockland, ME) with 1% SDS (Gibco by Invitrogen, Grand Island, NY) and embedded in plug molds (BioRad, Hercules, CA). The plugs were digested with EC buffer (6mM Tris chloride [pH 7.6], 1M NaCl, 100mM EDTA [pH 7.6], 1% sarcosyl, 1 mg/ml lysozyme) (Sigma Aldrich, St. Louis, MO) overnight at 37°C with constant shaking. The digested plugs were then lysed by immersing in 5 ml of lysis buffer [(50mM Tris:50mM EDTA, pH 8.0 with 1% sarcosyl) containing 0.1 mg/ml Proteinase K] at 54°C overnight with constant shaking. The plugs were washed twice in molecular grade water (Fisher, Pittsburgh, PA) with gentle shaking for 15 min at 50°C. This was followed by two washes in TE buffer (10 mM Tris:1 mM EDTA, pH 8) containing 1mM phenylmethylsulphonyl fluoride (PMSF) (Sigma Aldrich St. Louis, MO) with gentle shaking for 30 min per wash; followed by four subsequent washes in TE buffer (10 mM Tris:1mM EDTA, pH 8.0) with gentle shaking for 15 min per wash.

**DNA restriction for PFGE.** Prior to restriction, a 3.0-4.0 mm plug was placed in 200 µl of 1:10 dilution of the restriction enzyme buffer (New England Biolabs, Beverly, MA) and ultra pure distilled water (Gibco, Grand Island, NY) incubated at room temperature for 15 min. The enzyme digestion was performed with a 1:10 dilution of restriction enzyme buffer and ultra pure water with 20U of Not I restriction endonuclease (New England Biolabs, Beverly, MA) and incubated overnight at 37°C.

**PFGE.** Restricted DNA fragments were separated in 1% SeaKem Gold agarose in 0.5X TBE using CHEF Mapper (BioRad, Richmond, CA) at 14°C for 14h. The initial switch time was 0.5 s with a final switch time of 40.01 s. The voltage gradient was 6.0 V/cm, 120° angle and a linear ramping factor.

**Preparation of crude supernatant fluid (CSF).** Supernatants from LAB with antimicrobial activity were concentrated for further analysis using the modified method by Hernandez et al 2005. LAB strains were grown in MRS broth at 37°C for 48 h under anaerobic conditions in order to eliminate the formation of H<sub>2</sub>O<sub>2</sub>. Aliquots of the cultures were centrifuged at 10, 000 x g for 15 min at 4°C. The supernatants were adjusted to pH 6.5 with 1M NaOH and filtered using 0.22 µm filter (Fischer Scientific, Pittsburg, PA). The pH adjusted cell free supernatants were then concentrated in a speed vacuum at 4°C (Sorvant Instruments). The concentrate obtained was designed as crude supernatant fluid (CSF) and was stored at -20°C until further use.

**Detection of inhibitory activity.** The agar well diffusion assay (AWDA) by Tag and McGiven (1971) was used without any modifications in assessing the inhibitory

activity of the CSF. Indicator strains in Table 1 were used in determining the inhibitory activity of the LAB isolates.

**Table 1: Bacterial strains and culture conditions\***

Species	Origin (No.)	Temperature (°C)	Medium, incubation
<b>Reference bacteriocin producing strains</b>			
<i>Pediococcus pentosaceus</i>	ATCC 33316	37	MRS, anaerobic
<i>Lactobacillus acidophilus</i>	ATCC 11975	37	MRS, anaerobic
<i>Lactoacillus rhamnosus</i>	ATCC 7469	37	MRS, anaerobic
<b>Indicator strains</b>			
<b>Gram - positive bacteria</b>			
<i>Bacillus cereus</i>	Culture collection	37	BHI, aerobic
<i>Micrococcus luteus</i>	Culture collection	37	BHI, aerobic
<i>Staphylococcus aureus</i>	ATCC 25293	37	BHI, aerobic
<i>Listeria monocytogenes</i>	ATCC 7644	37	BHI, aerobic
<i>Enterococcus faecalis</i>	ATCC 29212	37	BHI, aerobic
<i>Enterococcus faecium</i>	Culture collection	37	BHI, aerobic
<i>Streptococcus pyogenes</i>	Culture collection	37	BHI, aerobic
<b>Gram - negative bacteria</b>			
<i>Escherichia coli</i> O157: H7	ATCC 35150	37	BHI, aerobic
<i>Enterobacter aerogenes</i>	Culture collection	37	BHI, aerobic
<i>Pseudomonas aeruginosa</i>	Culture collection	37	BHI, aerobic
<i>Serratia marcescens</i>	Culture collection	37	BHI, aerobic
<i>Shigella sonnei</i>	ATCC 9290	37	BHI, aerobic
<i>Salmonella typhi</i>	ATCC 4161	37	BHI, aerobic
<b>Yeast</b>			
<i>Saccharomyces cerevisiae</i>	ATCC 9763	30	YPD, aerobic

\*ATCC, American Type Culture Collection (Manassas, VA); culture collection, Dr. B. E. Eribo (Howard University, Department of Biology).

**Enzyme sensitivity.** In order to determine the sensitivity to enzymes, the CSFs were treated with the following enzymes:  $\alpha$ -chymotrypsin, trypsin, pepsin, lipase A,

amylase, protease XIV and proteinase K (Sigma Aldrich St. Louis, MO) according to the method used by Hernandez et al. (2005).

**Sensitivity of bacteriocin to heat.** The CSF was exposed to the following heat treatments: 100°C for 2 h, 4 h, 6.5 h and 121°C for 45 min. After the samples were allowed to cool, activity was determined by AWDA.

**Table 2: Primers used for identification of genes encoding bacteriocin production**

Target gene	Designation	Sequence (5' - 3')	Amplicon size (bp)	Reference
Pediocin	PedF	GGTAAGGCTACCACCTGTCAT	332	Suwanjinda et al. (2007)
	PedR	CTACTAACGCTTGCTGGCA		
Pediocin PA-1/AcH	EDRPO	CAAGATCGTTAACCAGTTT	1044	Todorov et al. (2010)
	PEDC1041	CCGTTGTTCCCATAGTCTAA		

**Identification of genes encoding the bacteriocin production.** DNA template was isolated by the boiling method. An exponential phase culture was grown for 24 h in MRS broth. The culture was centrifuged at 14,000 x g for 10 min at 4°C. The cell pellets were resuspended in 200 ul of distilled water. The cell suspension was subjected to boiling for 10 min and centrifugation as described above. The Primers PEDRPO, PEDC1041, PedF and PedR were obtained from Invitrogen and are listed in Table 2. PCR

reactions were performed using MJ Mini Gradient Thermal cycler (Biorad). The PCR analysis was carried out using Econotaq master mix (Lucigen Corporation, Middleton, WI). PCR amplification was conducted using 1 ul of the DNA template, 25 ul of the master mix containing a combined volume of 2 ul primers, for a total volume of 50 ul. The amplification parameters were as follows: 1 min at 94 °C initial denaturation to activate Econotaq, 30 cycles that consisted of a 1 min denaturation step at 94 °C, 30 s annealing step at 50 °C, 45 s elongation step at 72 °C and a final extension step of 5 min. The amplified products were analyzed using a 1.2% (w/v) agarose gel.

**DNA sequencing of the bacteriocin-encoding genes.** Amplified DNA from agarose gel was purified by the use of Qiagen gel extraction kit (Qiagen Inc, Valencia, CA). The purified PCR product was subjected to DNA sequencing by Genewiz Maryland Laboratory, USA. Similarity searches to the sequences obtained were performed with BLAST program (<http://www.ncbi.nlm.nih.gov>).

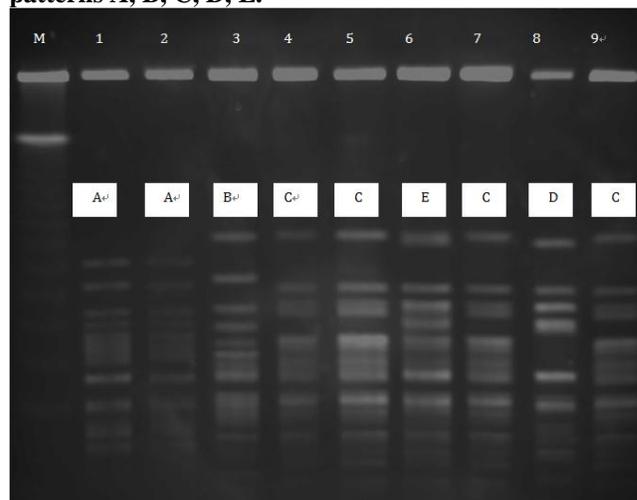
## Results

**Screening for antimicrobial activity.** Three hundred non-starter lactic acid bacteria were isolated from retail cheddar cheese and they were screened for antimicrobial activity using the agar spot test method. Of these, 130 isolates were found to have inhibitory activity against *S. aureus* and *E. coli* O157:H7. Inhibition due to acid and hydrogen peroxide were excluded by neutralization with NaOH and treatment of the supernatants with catalase. From the 130 isolates, 80 were *Lactobacillus* strains and 50 were *Pediococcus* strains. Since this study was focused on bacteriocin production by non-starter *Lactobacillus* strains, eight representative isolates showing strong antilisterial activity were selected for further studies. These isolates were named MC19, MC96, MC53, MC42, MC1, MC49, MC118 and MC72.

**Identification of the isolates.** API 50CH identified six of the eight isolates as *Lactobacillus paracasei* and the remaining two as *Lactobacillus brevis*. In order to validate the physiological and biochemical tests obtained by API 50CH, 16S rRNA sequencing was performed. Analysis of 16S rRNA sequencing results identified four isolates as *L. paracasei*, three as *Lactobacillus casei* and one as *Lactobacillus rhamnosus* (Table 3).

**PFGE.** Pulse field gel electrophoresis (PFGE) of Not I digested genomic DNA of bacteriocin producing *Lactobacillus* strains (MC1, MC19, MC72, MC42, MC49, MC53, MC96 and MC118) from Cheddar cheese distinguished 5 different banding patterns (Figure 1).

**Figure 1: PFGE patterns of NotI digests of genomic DNA of different bacteriocin producing *Lactobacillus* strains.** Lane M, Lambda Ladder (Bio-Rad); lane 1, *L. acidophilus* ATCC 11975; lane 2, MC42; lane 3, MC1; lane 4, MC19; lane 5, MC72; lane 6, MC49; lane 7, MC53; lane 8, MC96 and lane 9, MC118. PFGE patterns A, B, C, D, E.



**Table 3: Phenotypic and molecular characterization of bacteriocin producing *Lactobacillus* strains isolated from Cheddar cheese and ATTC reference strain.**

Strains	Phenotypic characterization*	Molecular characterization <sup>1</sup>
<i>Lactobacillus rhamnosus</i> ATCC 7469	<i>L. rhamnosus</i> (100%)	<i>L. rhamnosus</i> (99%)
MC96	<i>Lactobacillus paracasei</i> (96%)	<i>Lactobacillus paracasei</i> (98%)
MC118	<i>L. paracasei</i> (92.5%)	<i>L. paracasei</i> (99%)
MC72	<i>L. paracasei</i> (99.3%)	<i>L. paracasei</i> (99%)
MC1	<i>Lactobacillus brevis</i> (71.2%)	<i>Lactobacillus casei</i> (99%)
MC53	<i>L. paracasei</i> (99.9%)	<i>L. paracasei</i> (99%)
MC49	<i>L. paracasei</i> (99.9%)	<i>L. casei</i> (99%)
MC19	<i>L. paracasei</i> (99.9%)	<i>L. casei</i> (99%)
MC42	<i>L. brevis</i> (95%)	<i>L. rhamnosus</i> (99%)

<sup>1</sup> 16S rRNA sequencing was done to characterize the isolates; (%), percentage maximum identity as shown in blast search.

\*phenotypic characterization using API 50CH; (%), percentage identity as shown by APIweb software

Table 4: Antibacterial spectrum of bacteriocin producing *Lactobacillus* strains from Cheddar cheese\*

Indicator strains	Isolated strains								ATCC strains	
	1	2	3	4	5	6	7	8	7469	33316
<b>Gram-positive</b>										
<i>B. cereus</i>	++	+++	+++	++	-	++	-	++	++	++
<i>M. luteus</i>	++	++	+++	+++	+++	++	+++	+++	+++	+++
<i>S.aureus</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>L. monocytogenes</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>E. faecalis</i>	++	+++	+++	++	++	++	++	++	++	+++
<i>E. faecium</i>	++	+++	+++	++	++	++	++	++	++	+++
<i>S. pyogenes</i>	++	++	++	++	++	+	+	+	++	++
<b>Gram -negative</b>										
<i>E. coli</i> O157: H7	+	++	++	+	+++	+++	++	++	++	++
<i>P. aeruginosa</i>	+	+++	++	+	++	++	+	++	++	++
<i>S. marcescens</i>	+	++	++	+	+	-	-	+	++	++
<i>S. sonnei</i>	++	++	++	+	++	+	-	+	+	+
<i>S. typhi</i>	+	++	++	+	++	+	-	+	++	-
<i>E.aerogenes</i>	+	++	+++	+	++	-	++	-	++	++
<b>Yeast</b>										
<i>Saccharomyces cerevisiae</i>	-	-	-	-	-	-	-	-	-	-

\*1-8 represent isolates MC42, MC96, MC118, MC72, MC53, MC49, MC19, MC42 and MC1. 7469, 33316 correspond to ATCC strains *L. rhamnosus* and *P. pentosaceus*. -, no inhibition (less than 5 mm); +, 5 mm < zone < 10mm; ++, 10 < zone < 15; +++, >15 mm.

**Detection of inhibitory activity.** All eight isolates showed a broad spectrum of activity as they displayed activity against both Gram-positive and Gram-negative indicator strains but not *Saccharomyces cerevisiae*. Three of the isolates (MC96, MC118 and MC1) were highly active against *Enterococcus Faecalis* and *E. faecium*. (Table 4).

**Effect of enzymes and temperature on antimicrobial agents.** The antimicrobial agents from the eight selected isolates were not affected by any of the enzymes tested except for Proteinase K, which resulted in partial inactivation of the antimicrobial agents. Also boiling for 6.5 h and autoclaving at 121°C for 45 minutes, and storage at -20 °C, 4 °C, 25 °C, 37 °C, and 45 °C for 30 days did not affect the activity.

**Identification of genes encoding the bacteriocin production.** PCR analysis using pediocin primers (PedF and PedR) identified a 322 bp fragment corresponding to pediocin in all the isolates (Figure 2). Furthermore, PCR amplification with PEDRPO and PEDC1041 rendered positive results for the presence of a 1044 bp DNA fragment, corresponding in size to the structural gene of pediocin PA-1 in the eight selected isolates (Figure 3).

Figure 2: PCR amplification fragments from bacteriocin producing *Lactobacillus* strains, using primer pairs Ped F and Ped R targeting pediocin bacteriocin. Lane M, 1kb plus DNA Ladder; lane 1, MC1; lane 2, MC19; lane 3, MC49; lane 4, MC53; lane 5, MC72; lane 6; MC118; Lane 7, MC20; lane 8, MC42; lane 9, MC96; lane 10, positive control; lane 11, negative control; lane 12, empty space and lane 13, empty space.

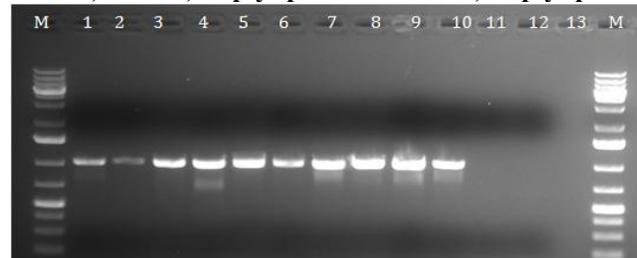
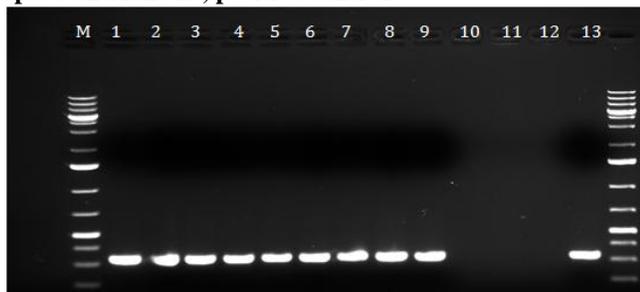


Figure 3: PCR products amplified using primers (PEDRO and PEDC1041) targeting bacteriocin pediocin PA-1 in *Lactobacillus* strains. Lane M, 1kb plus DNA Ladder; lane 1, MC1; lane 2, MC19; lane 3, MC49; lane 4, MC53; lane 5, MC72; lane 6; MC118; Lane 7, MC20; lane 8, MC42; lane 9, MC96; lane 10,

negative control; lane 11, empty space; lane 12, empty space and lane 13, positive control.



**DNA sequencing of the bacteriocin-encoding genes.** The 1044 bp fragments of MC1, MC19, MC49, MC53, MC42, MC118, MC72 and MC96 contained *pedA*, *pedB* and part of *pedC* genes. Sequence analysis of 1044 bp fragments of all the isolates revealed 99% homology with the *Lactobacillus plantarum* plasmid pWHE92 pediocin AcH production operon (Genebank accession no. AY316526).

### Discussion

In this study, we isolated several non-starter bacteriocin producing lactic acid bacterial population in retail Cheddar cheese. However, only eight representative *Lactobacillus* strains were selected for further studies based on their high antilisterial activity. Varied results were obtained from the phenotypic identification with API 50CH and 16S rRNA sequencing. This variation may be due to the fact that API 50CH is based on the selection of phenotypic markers whose expression relies on environmental conditions (Fitzsimons et al. 1999). Similar observation was made by Fitzsimons et al. (1999). In general, the *Lactobacillus* strains were heterogenous as further confirmed by pulse field gel electrophoresis (PFGE) which is an important tool in the analysis of bacterial genome of lactic acid bacteria (LAB).

The antimicrobial agents of the non-starter bacterial isolates displayed typical characteristics of bacteriocins; neutralization with NaOH had no effect on their activity indicating that lactic acid was not responsible for the activity, lipase A and amylase also had no effect suggesting that the active moiety had no lipid component neither was it glycosylated. The observed partial inactivation of the antimicrobial agents by proteinase K is indicative of their proteinaceous nature. Unlike most bacteriocins, which are typically susceptible to the activities of such proteolytic enzymes as pepsin, trypsin, protease XIV and  $\alpha$ -chymotrypsin, bacteriocins in this study remained active after treatment with the enzymes. A similar pattern of proteolytic enzyme activity was observed with pediocin SA-1 isolated from *Pediococcus acidilactici* NRRL B5627 (Anastasiadou et al. 2008). To the best of our knowledge,

this is the first report of such bacteriocin in non starter *Lactobacillus* strains.

The bacteriocins in this study had broad spectrum of activity as they inhibited both Gram-negative and Gram-positive bacteria foodborne pathogens including *Bacillus cereus*. Of particular interest was their strong antilisterial activity, which is a characteristic of Class IIa bacteriocins. A desirable characteristic of bacteriocins isolated in this study is their heat stability as they were resistant to autoclaving at 121°C for 45 minutes and boiling for up to 6.5 h, which make them suitable for application in pasteurized foods.

Further characterization of the bacteriocin producing isolates by PCR amplification analysis identified a 1044 bp fragment corresponding to pediocin PA-1/AcH. Pediocin PA-1/AcH biosynthesis involves a DNA fragment of approximately 3.5 kb, comprising of four genes *pedA*, *pedB*, *pedC* and *pedD* (Marugg et al. 1992). The presence of *pedA*, *pedB*, and part of *pedC* genes in all eight *Lactobacillus* strains confirmed that the bacteriocin produced by these isolates was pediocin PA-1-like. Pediocin PA-1 is a class IIa bacteriocin that has been well studied (Venema et al. 1995) and it has been associated with strong activity against *Listeria monocytogenes*, a foodborne pathogen of concern in cheese. However, it was of interest that *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* were found to be pediocin PA-1/AcH producers as these have not previously been known to produce this bacteriocin. Although there was 99% similarity between the bacteriocin structural gene of the isolates in this study and *Lactobacillus plantarum* pWHE92 pediocin AcH production operon, the bacteriocins in this study were found to be of broad-spectrum in comparison to the latter that is narrow spectrum.

Pediocin PA-1-like bacteriocin produced by the non-starter lactic acid bacteria in this study is a promising candidate for biopreservation in the dairy industry. This study is an important development for the dairy industry since most of the strains that normally produce this bacteriocin are of meat and vegetable origin.

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