ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

BIOPRESERVATIVE POTENTIAL AND STABILITY ASSESSMENT OF PEDIOCOCCUS ACIDILACTICI BA28

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Manuscript Info

Manuscript History:

Received: 15 July 2015
Final Accepted: 14 August 2015
Published Online: September 2015

Key words:

Pediococcus acidilactici, Pediocin BA28, Food Biopreservatives, Bacteriocin

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Abstract

Bacteriocins are generally recognized as "natural" compounds able to influence the safety and quality of foods. In the past years, a lot of works have been aimed to the detection, purification and characterisation of bacteriocins, as well as to their use in food preservation strategies. Several LAB bacteriocins offer potential applications in food preservation, and the use of bacteriocins in the food industry can help to reduce the addition of chemical preservatives as well as the intensity of heat treatments, resulting in foods which are more naturally preserved and richer in organoleptic and nutritional properties. This can be an alternative to satisfy the increasing consumers demands for safe, fresh-tasting, ready-to-eat, minimally-processed foods and also to develop "novel" food products. Lactic acid bacteria and their antimicrobial metabolites have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods.

INTRODUCTION

Bio-preservation has gained increasing attention as natural means for controlling the shelf life and safety of food products. The application of bio-protective cultures to ensure the hygienic quality is a promising tool although, it should be considered only as an additional measure to good manufacturing, processing, and storage and distribution practices (Holzapfel et al., 1995). Lactic acid bacteria (LAB) have shown a major potential for use in bio-preservation because of safety for human consumption (GRAS status) and the prevalent microflora during storage in many foods (Vignolo et al., 2008). LAB produces wide range of antimicrobial metabolites, i.e. organic acids, diacetyl, acetoin, hydrogen peroxide and bacteriocins. These antimicrobial activities can contribute in the microbiological safety by controlling the growth of other microorganisms and inhibition of pathogenic bacteria involved in spoilage of food products (Caplice and Fitzgerald, 1999).

The nutritious and therapeutic benefits of probiotic microorganisms have been most extensively investigated in dairy products such as milk, yogurt (Khalil and Mansour, 1998) and cheese (Ong et al., 2006; Meyer et al., 2007). Bacteriocins of LAB have great commercial potential as natural food preservatives due to their highly selective antimicrobial activities (Cotter *et al*., 2005). They are cationic small, ribosomally synthesized, secretary peptides or proteins which may exhibit bactericidal or bacteriostatic effect on sensitive bacteria (Klaenhammer, 1988). With the discovery of colicin by Gratia in 1925, LAB producing bacteriocin has attracted great interest in terms of food safety, due to their "generally recognised as safe" (GRAS) status.

Pediocin BA28 belongs to class IIa bacteriocin family, purified and characterized from natural isolate *Pediococcus acidilactici* BA28 (Kaur et al., 2012; Kaur and Garg, 2013). It possesses a wide range of antimicrobial spectrum against various pathogenic microorganism (including many species of *Aspergillus, Bacteroides, Clostridium, Escherichia, Enterococcus, Gardenerella, Helicobacter, Klebsiella, Lactobacillus, Leuconostoc, Listeria, Micrococcus, Neisseria, Pediococcus, Pseudomonas, Propionibacterium, Proteus, Staphylococcus, Streptococcus*

and *Vibrio*) that raised the possibility of its potential as an ingredient of food preservation and medical and/or personal care products. Keeping in view the requirement of GRAS preservatives, this study was undertaken with the aim to explore stability and bio-preservative potential of Pediocin BA28 in some model food systems.

MATERIALS AND METHODS

Bacterial strains and growth conditions: A bacteriocin (Pediocin BA28), produced by *Pediococcus acidilactici* BA28 strain, was isolated by pour plate technique from fecal samples taken with informed consent from healthy human volunteers. Antimicrobial spectrum of pediocin BA28 was determined by spot-on-lawn assay against *P. acidilactici* LB42 and *E. fecalis* NDRI isolate used as indicator strains (Coventry *et al.*, 1995).

Partial purification of bacteriocin: Overnight grown culture of *P. acidilactici* BA28 in MRS medium (supplemented with 0.1% w/v Tween-80; pH 6.5) was used for preparation of purified bacteriocin by conventional adsorption-desorption method (Yang et al., 1992; Kaur and Garg, 2013). Bacteriocin preparation was then filter sterilized using 0.45 µm filters (Millipore, USA).

Bacteriocin activity assay: The antimicrobial activity of bacteriocin preparation was confirmed by well diffusion assay according to the protocol of Cintas *et al.* (1998) and bacteriocin activity was calculated as arbitrary unit (AU) and expressed as AU/ml as per standard protocol of Pucci *et al.* (1988). Spot-on-lawn assays were performed using 5µl of each dilution against *P. acidilactici* LB42 and *E. fecalis* NDRI isolate as reference microorganisms for the determination of a bacteriocin's biological activity.

Biopreservative potential and stability of bacteriocin in different model food systems: Bacteriocin degradation was studied in different model food systems like pasteurized milk, vegetable fresh cuts, moong sprouts, channa sprouts and minced meat obtained from various retail shops. Four screw capped sterilized glass vials were taken. 10 ml of 100 mM saline was added in these vials. Then the vials were sterilized by autoclaving at 15 psi for 20 min. Different concentration of purified bacteriocin (5000 AU/ml, 10,000 AU/ml and 20,000 AU/ml) were added to the vials. 10 g of samples were added in the vials and were properly mixed. Stability of bacteriocin was also tested in 10 ml pasteurized milk sample. Samples were preserved at 4 ºC. After a regular interval of 10 days, samples from each vial were collected to enumerate microbial counts on MRS agar, Nutrient agar, *Listeria* Enrichment broth, EMB agar, SS HiVeg agar, Rapport Vassiliadis *Salmonella* Enrichment Broth and *Clostridial* Reinforced media. All the samples were assayed to check the residual bacteriocin activity by spot-on-lawn method and microbial counts as cfu/ml (Joshi *et al.*, 2006).

Statistical analysis: Where ever appropriate, experimental data are expressed as mean values and standard deviations. Statistical significance of the results were tested by one way ANOVA. A probability value of $p>0{\cdot}05$ was used as the criterion for statistical significance.

RESULTS

In the present study, pediocin BA28 produced by *P. acidilactici* BA28 was found to possess wide antibacterial activity determined by the Disc diffusion method and spot-on-lawn assay. Pediocin BA28 is capable of inhibiting a number of human pathogens including *B. fragilis, B. ovatus, B. vulgatus,* yeast *C. albicans, C. sporogenes, E. coli, E. faecalis, G. vaginalis, H. pylori, K. pneumoniae, L. mesenteroides, L. monocytogenes, M. flavus, N. gonorroeae, N. mucosa, P. aeruginosa, P. mirabilis, Staphylococci, Streptococci, , S. typhi* and *V. cholerae.* It did not show any activity against *B. subtilis, C. perfringens, L. casei*, *L. leichmanni*, *L. plantarum*, *L. pentosus*, *L. lactis* subsp. *cremoris, S. agalactiae* and *Y.* enterocoloitica (Kaur et al., 2012).

An increase in the antimicrobial activity after the partial purification of crude bacteriocin by conventional adsorption-desorption method is observed. The antimicrobial activity of the purified bacteriocin in terms of the diameter of zone of inhibition increased from 16mm to 27mm. There was 2.69 fold increase in the partially purified bacteriocin activity than that of the crude bacteriocin (Kaur and Garg, 2013).

Biopreservation potential and stability of bacteriocin in different model food systems:

Bacteriocin i.e. pediocin BA28 degradation was studied in different model food systems as given below:

1. **Pasteurized milk sample**: Pediocin activity showed a little reduction in the treated milk samples stored upto 10 days. However, it was observed to decrease with time and was completely vanished at 30th day of incubation under refrigerated conditions that may be due to degradation of bacteriocin by complex interplay of components of food matrix used. Microbial counts ($\log_{10}$ cfu/ml) were observed on different media as mentioned above and results were recorded in **tables 1**. Pediocin BA28 was found to be less effective against other lactic acid bacteria. Though, with increase in bacteriocin concentration, a slight reduction in microbial counts was observed. Pediocin BA28 produced by *Pediococcus acidilactici* BA28 was found to possess very strong anti-*listerial* activity. Coliforms and *Clostridial* counts ($\log_{10}$ cfu/ml) also decreased with increased pediocin

concentration. Antimicrobial effect remained stable for 20 days of storage at refrigeration temperature. Higher concentration of pediocin BA28 (20,000 AU/ml) was required to inhibit the growth of pathogenic microorganisms such as *Salmonella* and *Shigella*. Thus, we can conclude that pediocin BA28 increases the shelf life of pasteurized milk when used at higher concentrations.

2. **Vegetable fresh cuts:** The results indicate approximately 90% recoverable pediocin activity after 10 days of addition to vegetables fresh cuts. It was however, degraded completely after 30 days of incubation at 4 ºC. Higher concentration of pediocin BA28 was required to inhibit *Salmonella* and *Shigella* (20,000 AU/ml) upto 20 days, while it was observed to be very effective in controlling growth of *Coliforms* and *Clostridia* even at a lesser concentration of 10,000 AU/ml. Anti-*listerial* activity of pediocin BA28 was stable upto 30 days of incubation at refrigeration temperature. Results therefore reflect the stability of pediocin BA28 to increase the shelf life of the vegetables fresh cuts upto 20 days provided it is used at a concentration more than 20,000 AU/ml **(Table 2)**.
3. **Moong sprouts:** A reduction in pediocin activity with increase in incubation time and it was completely lost at 20th day of incubation. Pediocin BA28 was observed to be less stable in moong sprouts as compared to the other food systems. Total microbial load present in the moong sprouts sample decreased with increase in the concentration of pediocin BA28 (**Table 3**). Pediocin BA28 has potential to inhibit the growth of *Coliforms* upto 20 days of preservation at refrigerated temperature. Antagonistic activity of pediocin against other lactic acid bacteria was poorly observed. Pediocin BA28 was found to effectively inhibit growth of *Listeria, Salmonella, Shigella* and *Clostidia* upto 20 days of preservation when used at concentration 20,000 AU/ml. Pediocin BA28 produced by *P. acidilactici* BA28 has broad spectrum of activity and has shown to increase the shelf life of moong sprouts. Because of degradation of pediocin BA28 in moong bean samples relatively higher concentrations of bacteriocin are required for their preservation than for other food systems.
4. **Channa sprouts:** Approximately 90% pediocin activity was recoverable after 10 days of addition to channa sprouts which was reduced to 50% after 20 days of storage of sprouts at 4 ºC. It was degraded completely after 30 days of incubation at 4 ºC. The microorganisms present (log_{10} cfu/ml) in pediocin treated channa sprouts were observed in stored samples as shown in **tables 4**. Bactericidal effect of pediocin BA28 against *Listeria monocytogenes* was significant and stable upto 20 days. As the concentration of pediocin BA28 was increased, a concordant decrease in total microbial load was observed. Pediocin at a concentration of 20,000 AU/ml was able to inhibit the growth of coliforms upto 30 days of preservation, whereas lower concentration 5,000 AU/ml was observed to inhibit their growth upto 20 days. It showed a poor antagonism against other lactic acid bacteria whose count showed a constant increase with increase in incubation time. Inhibition of pathogenic microorganisms *Salmonella* and *Shigella* was observed upto 20 days of preservation at refrigerated temperatures when pediocin BA28 was used at higher concentrations. Thus, pediocin BA28 antagonism was more prominent during initial days of incubation.
5. **Minced meat:** Pediocin activity was observed to decrease with time and was reduced to 25%, 53% and 0% after 10, 20 and 30 days of incubation respectively under refrigeration conditions. Biopreservation of pediocin BA28 treated minced meat samples were studied during storage at 4 ºC. It was found to inhibit the growth *Salmonella* and *Shigella* upto 20 days of preservation. Anti-*listerial* activity of pediocin was prominent till 30th day of preservation when used at concentration 20,000 AU/ml whereas when used at lower concentrations *listerial* growth was inhibited upto 20 days of preservation. Lower concentrations of pediocin BA28 were sufficient to inhibit the growth of coliforms while higher concentration was required to inhibit the *Clostridial* counts in the food samples (**Table 5**). It was found to inhibit the growth of other lactic acid bacteria but to a lesser extent.

Table 1: Microbial counts (log_{10} cfu/ml) in pasteurized milk sample

Time (days)	Control	Test samples treated with bacteriocin (AU/ml)		
		5000	10,000	20,000
MRS Agar				
0	5.161 ± 0.021	5.093 ± 0.200	5.068 ± 0.127	4.991 ± 0.093
10	6.311 ± 0.153	6.260 ± 0.100	6.220 ± 0.109	6.113 ± 0.130
20	6.389 ± 0.093	6.315 ± 0.069	6.260 ± 0.205	6.204 ± 0.128
30	6.480 ± 0.115	6.451 ± 0.035	6.414 ± 0.214	6.320 ± 0.108
Nutrient Agar				
0	4.826 ± 0.180	4.763 ± 0.079	4.724 ±0.115	4.602 ± 0.075

10	6.113 ± 0.110	6.100 ± 0.040	5.913 ± 0.134	5.778 ± 0.083
20	6.217 ± 0.152	6.152 ± 0.056	6.093 ± 0.069	5.880 ± 0.014
30	6.389 ± 0.130	6.354 ± 0.205	6.294 ± 0.160	5.977 ± 0.105
EMB Agar				
0	3.301 ± 0.004	-ve	-ve	-ve
10	4.001 ± 0.137	-ve	-ve	-ve
20	4.301 ± 0.070	4.001 ± 0.087	-ve	-ve
30	4.602 ± 0.042	4.477 ± 0.099	4.001 ± 0.151	-ve
***Listeria* Enrichment Agar**				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	-ve	-ve	-ve	-ve
30	4.602 ± 0.131	4.477 ± 0.094	4.001 ± 0.112	-ve
***Clostridial* Reinforced Agar**				
0	5.826 ± 0.020	5.176 ± 0.120	-ve	-ve
10	5.954 ± 0.059	5.342 ± 0.109	-ve	-ve
20	5.991 ± 0.100	5.633 ± 0.073	-ve	-ve
30	6.068 ± 0.045	5.944 ± 0.105	5.662 ± 0.148	5.447 ± 0.122
SSHiveg Agar				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.778 ± 0.166	4.602 ± 0.169	4.477 ± 0.078	-ve
30	4.954 ± 0.219	4.778 ± 0.113	4.698 ± 0.150	4.301 ± 0.130
Rapport Vassiliadis *Salmonella* Enrichment Broth				
0	5.806 ± 0.076	5.653 ± 0.101	5.079 ± 0.111	-ve
10	5.973 ± 0.111	5.748 ± 0.072	5.361 ± 0.021	-ve
20	6.292 ± 0.153	5.982 ± 0.115	5.924 ± 0.110	-ve
30	6.320 ± 0.093	6.278 ± 0.180	6.33 ±0.152	5.653±0.130
Data is an average of two independent experiments; p value= 1.62E-31;α (level of significant)=0.05; F crit=1.548 and F value=39.890				

Table 2: Microbial counts ($\log_{10}$ cfu/ml) in vegetables fresh cuts

Time (days)	**Control**	**Test samples treated with bacteriocin (AU/ml)**		
		5000	**10,000**	**20,000**
MRS Agar				
0	4.826 ± 0.093	4.763 ± 0.091	4.724 ± 0.188	4.602 ± 0.200
10	6.113 ± 0.106	6.100 ± 0.115	5.913 ± 0.088	5.778 ± 0.104
20	6.217 ± 0.110	6.152 ± 0.081	6.093 ± 0.231	5.880 ± 0.124
30	6.389 ± 0.109	6.354 ± 0.099	6.294 ± 0.168	5.977 ± 0.077
Nutrient Agar				
0	4.672 ± 0.101	4.643 ± 0.108	4.579 ± 0.226	4.544 ± 0.168
10	6.161 ± 0.159	6.110 ± 0.101	5.949 ± 0.201	5.812 ± 0.068
20	6.230 ± 0.093	6.161 ± 0.112	6.110 ± 0.169	5.949 ± 0.227
30	6.439 ± 0.110	6.371 ± 0.095	6.313 ± 0.107	6.294 ± 0.200

EMB Agar				
0	4.001 ± 0.095	-ve	-ve	-ve
10	4.301 ± 0.137	-ve	-ve	-ve
20	4.477 ± 0.133	4.301 ± 0.291	-ve	-ve
30	4.602 ± 0.158	4.477 ± 0.101	4.301 ± 0.094	-ve
***Listeria* Enrichment Agar**				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	-ve	-ve	-ve	-ve
30	4.477 ± 0.096	4.301 ± 0.080	4.001 ± 0.110	-ve
***Clostridial* Reinforced Agar**				
0	5.812 ± 0.099	-ve	-ve	-ve
10	5.977 ± 0.056	5.301 ± 0.189	-ve	-ve
20	5.982 ± 0.103	5.698 ± 0.077	-ve	-ve
30	6.060 ± 0.147	5.991 ± 0.041	5.748 ± 0.127	5.623 ± 0.157
SSHiveg Agar				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.845 ± 0.119	4.778 ± 0.098	4.698 ± 0.104	-ve
30	5.041 ± 0.088	4.954 ± 0.118	4.903 ± 0.113	4.301 ± 0.108
Rapport Vassiliadis *Salmonella* Enrichment Broth				
0	5.633 ± 0.145	5.531 ± 0.122	-ve	-ve
10	5.973 ± 0.107	5.748 ± 0.155	5.361 ± 0.122	-ve
20	6.292 ± 0.101	5.982 ± 0.115	5.924 ± 0.085	-ve
30	6.320 ± 0.074	6.278 ± 0.168	6.033 ± 0.099	5.653 ± 0.114
Data is an average of two independent experiments; p value= 1.62E-31;α (level of significant)=0.05; F crit=1.548 and F value=39.890				

Table 3: Microbial counts (log_{10} cfu/ml) in moong sprouts

Time (days)	**Control**	**Test samples treated with bacteriocin (AU/ml)**		
		5000	**10,000**	**20,000**
MRS Agar				
0	5.281 ± 0.084	5.217 ± 0.122	5.161 ± 0.078	5.103 ± 0.091
10	6.342 ± 0.223	6.245 ± 0.101	6.209 ± 0.127	6.100 ± 0.129
20	6.408 ± 0.174	6.311 ± 0.118	6.298 ± 0.118	6.187 ± 0.156
30	6.436 ± 0.116	6.416 ± 0.095	6.344 ± 0.110	6.296 ± 0.106
Nutrient Agar				
0	4.919 ± 0.114	4.954 ± 0.130	4.880 ± 0.079	4.792 ± 0.098
10	6.146 ± 0.166	6.096 ± 0.067	5.944 ± 0.109	5.716 ± 0.106
20	6.209 ± 0.107	6.173 ± 0.049	6.100 ± 0.808	5.934 ± 0.110
30	6.419 ± 0.073	6.376 ± 0.119	6.348 ± 0.097	6.271 ± 0.091
EMB Agar				
0	4.301 ± 0.270	-ve	-ve	-ve
10	4.001 ± 0.099	-ve	-ve	-ve
20	4.477 ± 0.159	-ve	-ve	-ve
30	4.602 ± 0.266	4.301 ± 0.185	4.001 ± 0.223	-ve
***Listeria* Enrichment Agar**				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.698 ± 0.213	4.477 ± 0.126	4.301 ± 0.129	-ve

30	5.204 ± 0.217	5.079 ± 0.168	5.041 ± 0.102	4.903 ± 0.122
***Clostridial* Reinforced Agar**				
0	5.672 ± 0.080	5.544 ± 0.127	-ve	-ve
10	5.913 ± 0.132	5.397 ± 0.128	-ve	-ve
20	6.176 ± 0.079	5.653 ± 0.116	-ve	-ve
30	6.227 ± 0.113	6.152 ± 0.110	6.107 ± 0.137	5.968 ± 0.318
SSHiveg Agar				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.698 ± 0.136	4.698 ± 0.106	4.698 ± 0.116	-ve
30	5.041 ± 0.089	4.903 ± 0.097	4.778 ± 0.089	4.602 ± 0.094
Rapport Vassiliadis *Salmonella* Enrichment Broth				
0	5.826 ± 0.134	5.806 ± 0.091	5.544 ± 0.113	-ve
10	5.934 ± 0.089	5.763 ± 0.086	5.447 ± 0.095	-ve
20	6.255 ± 0.073	5.913 ± 0.011	5.880 ± 0.101	5.633 ± 0.116
30	6.390 ± 0.067	6.359 ± 0.119	6.334 ± 0.122	6.107 ± 0.107
Data is an average of two independent experiments; p value= 1.62E-31;α (level of significant)=0.05; F crit=1.548 and F value=39.890				

Table 4: Microbial counts (log_{10} cfu/ml) in channa sprouts sample

Time (days)	**Control**	**Test samples treated with bacteriocin (AU/ml)**		
		5000	**10,000**	**20,000**
MRS Agar				
0	5.285 ± 0.067	5.222 ± 0.166	5.190 ± 0.112	5.110 ± 0.105
10	6.344 ± 0.136	6.260 ± 0.202	6.209 ± 0.146	6.079 ± 0.112
20	6.397 ± 0.118	6.320 ± 0.150	6.292 ± 0.164	6.209 ± 0.135
30	6.439 ± 0.020	6.426 ± 0.183	6.357 ± 0.134	6.305 ± 0.150
Nutrient Agar				
0	4.929 ± 0.112	4.913 ± 0.171	4.875 ± 0.105	4.832 ± 0.088
10	6.130 ± 0.107	6.082 ± 0.141	5.913 ± 0.068	5.707 ± 0.111
20	6.235 ± 0.014	6.120 ± 0.117	6.117 ± 0.109	5.913 ± 0.107
30	6.419 ± 0.105	6.394 ± 0.170	6.356 ± 0.112	6.290 ± 0.118
EMB Agar				
0	4.001 ± 0.114	-ve	-ve	-ve
10	4.301 ± 0.074	-ve	-ve	-ve
20	4.477 ± 0.076	-ve	-ve	-ve
30	4.602 ± 0.116	4.301 ± 0.090	4.001 ± 0.060	-ve
***Listeria* Enrichment Agar**				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	-ve	-ve	-ve	-ve
30	4.602 ± 0.110	4.301 ± 0.073	4.001 ± 0.112	-ve
***Clostridial* Reinforced Agar**				
0	5.732 ± 0.108	5.278 ± 0.116	-ve	-ve
10	5.973 ± 0.111	5.447 ± 0.113	-ve	-ve
20	6.146 ± 0.097	5.838 ± 0.108	-ve	-ve
30	6.276 ± 0.164	6.170 ± 0.076	6.120 ± 0.128	5.875 ± 0.087
SSHiveg Agar				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.698 ± 0.111	4.602 ± 0.141	4.698 ± 0.125	-ve
30	4.903 ± 0.074	4.845 ± 0.103	4.778 ± 0.159	4.301 ± 0.122
Rapport Vassiliadis *Salmonella* Enrichment Broth				
0	5.544 ± 0.103	5.380 ± 0.023	5.204 ± 0.141	-ve

10	5.963 ± 0.181	5.792 ± 0.098	5.414 ± 0.150	-ve
20	6.274 ± 0.121	5.934 ± 0.101	5.880 ± 0.202	-ve
30	6.313±0.094	6.271±0.111	6.021±0.183	5.929±0.088
Data is an average of two independent experiments; p value= 1.62E-31;α (level of significant)=0.05; F crit=1.548 and F value=39.890				

Table 5: Microbial counts (log_{10} cfu/ml) in minced meat sample

Time (days)	**Control**	**Test samples treated with bacteriocin (AU/ml)**		
		5000	**10,000**	**20,000**
MRS Agar				
0	5.292 ± 0.050	5.220 ± 0.182	5.161 ± 0.179	5.089 ± 0.335
10	6.322 ± 0.169	6.274 ± 0.114	6.181 ± 0.142	6.107 ± 0.067
20	6.365 ± 0.134	6.326 ± 0.195	6.274 ± 0.560	6.209 ± 0.246
30	6.439 ± 0.109	6.392 ± 0.124	6.376 ± 0.161	6.334 ± 0.512
Nutrient Agar				
0	4.875 ± 0.150	4.832 ± 0.170	4.732 ± 0.120	4.623 ± 0.190
10	6.158 ± 0.190	6.079 ± 0.115	5.924 ± 0.241	5.755 ± 0.211
20	6.255 ± 0.166	6.146 ± 0.204	6.096 ± 0.314	5.869 ± 0.222
30	6.423 ± 0.248	6.390 ± 0.210	6.336 ± 0.318	6.267 ± 0.079
EMB Agar				
0	2.477 ± 0.230	-ve	-ve	-ve
10	4.001 ± 0.165	-ve	-ve	-ve
20	4.301 ± 0.121	-ve	-ve	-ve
30	4.602 ± 0.151	4.301 ± 0.157	4.001 ± 0.263	-ve
***Listeria* Enrichment Agar**				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	-ve	-ve	-ve	-ve
30	4.477 ± 0.160	4.301 ± 0.125	4.001 ± 0.210	-ve
***Clostridial* Reinforced Agar**				
0	5.633 ± 0.114	5.531 ± 0.185	5.414 ± 0.171	-ve
10	5.913 ± 0.135	5.740 ± 0.114	5.544 ± 0.215	-ve
20	6.260 ± 0.175	5.954 ± 0.550	5.857 ± 0.166	-ve
30	6.320 ± 0.111	6.278 ± 0.151	6.033 ± 0.180	5.812 ± 0.361
SSHiveg Agar				
0	-ve	-ve	-ve	-ve
10	-ve	-ve	-ve	-ve
20	4.845 ± 0.316	4.778 ± 0.166	4.698 ± 0.137	-ve
30	4.954 ± 0.215	4.845 ± 0.145	4.778 ± 0.166	4.301 ± 0.165
Rapport Vassiliadis *Salmonella* Enrichment Broth				
0	5.653 ± 0.209	5.079 ± 0.169	-ve	-ve
10	5.934 ± 0.210	5.301 ± 0.134	-ve	-ve
20	6.230 ± 0.172	5.819 ± 0.109	-ve	-ve
30	6.271 ± 0.144	6.170 ± 0.182	6.193 ± 0.194	5.892 ± 0.124
Data is an average of two independent experiments; p value= 1.62E-31;α (level of significant)=0.05; F crit=1.548 and F value=39.890				

DISCUSSION

Biopreservative potential and stability of pediocin BA28 was tested in milk and vegetables fresh cuts that indicated its effectiveness against a wide range of microbial pathogens including *Listeria monocytogenes*, Coliforms, *Clostridia, Salmonella* and *Shigella*. Thus, we can conclude that pediocin BA28 is an effective biopreservative for milk and vegetables fresh cuts when used at optimal concentration. Many literature citations focus on the biopreservation of milk using bacteriocins of LAB (Kumar *et al.,* 2012). *L. monocytogenes* has been the documented cause of a number of outbreaks associated with dairy products, such as pasteurized milk (Fleming *et al.*, 1985) and

cheese (James *et al.*, 1985) and nisin has been shown effective against *L. monocytogenes* in dairy products. The synergistic effect of nisin and nitrite on delaying outbreaks of *L. monocytogenes* and *Leuconostoc mesenteroides* was studied by Gill and Holley (2003). Chelating agents permeate the outer membrane of Gram-negative bacteria by extracting Ca^{2+} and Mg^{2+} cations that stabilize lipopolysaccharides of this structure, allowing bacteriocins to reach the cytoplasmic membrane (Helander *et al.*, 1997). Various chelators such as EDTA, disodium pyrophosphate, trisodium phosphate, haxametaphosphate or citrate have shown the enhanced effect with nisin both under laboratory conditions and in foods (Stevens *et al.*, 1991).

There has been a great interest in the application of bacteriocins and bacteriocin-producing strains (especially those produced by the LAB) on the preservation of foods of animal origin, but to a much less extent on vegetable foods (Abriouel *et al.*, 2010). Bennik *et al.* (1999) tested two bacteriocin-producing *P. parvulus* strains (isolated from minimally-processed vegetables) and one *Enterococococcus mundtii* ATO6 strain (isolated from chicory endive) for the biopreservation of vegetables. Nisin and pediocin PA-1/AcH are already available on the market for application in processed foods. Nisin and pediocin solutions were tested as possible sanitizer treatments on cabbage and broccoli against a bacterial cocktail of five *L. monocytogenes* strains (Bari *et al.*, 2005).

Enterocin AS-48 can be produced economically on semi-synthetic media (Abriouel *et al.*, 2003) and on whey-based substrates (Ananou *et al.*, 2010), which makes this bacteriocin an amenable antimicrobial for application in foods. Because of its broad spectrum of inhibition and increased stability due to its cyclic structure, enterocin AS-48 can be a sound candidate for decontamination of vegetable foods containing *L. monocytogenes* and other foodborne bacteria sensitive to this bacteriocin.

Earlier study on mungbean sprouts dipped in a solution of purified mundticin ATO6 (200 AU/ml) or coated with an alginate film containing the bacteriocin (200 AU/ml), showed a two log-decline of *listerial* counts after treatments (Bennik *et al.*, 1999). The mundticin-alginate coating provided the best results, especially within days 5 and 10 of incubation. Nisin and pediocin were tested for the biopreservation of the sprouts (Bari *et al.*, 2005). Results from sprout samples treated with enterocin AS-48 revealed modifications of the microbial populations during storage, apparently increasing the proportion of *Enterococcus* and *Leuconostoc* bacteria and decreasing the levels of Gram-negative bacteria (such as *Pantoea*, *Escherichia* and *Enterobacter*) on the sprouts during storage (Cobo Molinos *et al.*, 2009).

In recent years, due to an increase in consumers' demand for mungbean, alfalfa, soybean, radish and other seed sprouts that are usually eaten raw in salads or in sandwiches, much work is being done for the biopreservation of the sprouts (Rosas and Escartin, 2000). Nisin and pediocin are the most thoroughly tested antimicrobials for the biopreservation of the sprouts (Bari *et al.*, 2005). Results from sprout samples treated with enterocin AS-48 revealed modifications of the microbial populations during storage, apparently increasing the proportion of *Enterococcus* and *Leuconostoc* and decreasing the levels of Gram-negative bacteria (such as *Pantoea*, *Escherichia* and *Enterobacter*) on the sprouts during storage (Cobo Molinos *et al.*, 2009). Our study has also indicated effectiveness of pediocin BA28 for biopreservative of channa sprouts and gave promising results when used at higher concentrations.

Many studies have been carried out to control *L. monocytogenes* in meat products since it is common within slaughterhouse and meat-packing environments and has been isolated from raw meat, cooked and ready-to-eat meat products (Ryser and Marth, 1999). The activity of nisin alone at concentrations of 400 and 800 IU/g and in combination with 2% sodium chloride against *L. monocytogenes* in minced raw buffalo meat was examined by Pawar and others (2000). Murray and Richard (1997) evaluated the anti-*listerial* activity of nisin A and pediocin AcH in decontamination of artificially contaminated pieces of raw pork.

Laukova and coworkers (1999) examined the effectiveness of enterocin CCM 4231 in controlling *L. monocytogenes* contamination in dry fermented Hornad salami. Schobitz and others (1999) determined the inhibitory capacity of a bacteriocin-like substance produced by *Carnobacterium piscicola* L103 against *L. monocytogenes* and found that the bacteriocin completely inhibited *L. monocytogenes* in vacuum-packaged meat after 14 days of storage at 4 °C. Vignolo *et al.* (1996) showed that lactocin 705 produced by *Lactobacillus casei* CRL 705 inhibited the growth of *L. monocytogenes* in ground beef. Degnan *et al.* (1992) demonstrated the possibility of using bacteriocinogenic cultures of *P. acidilactici* (pediocin AcH producer) to control *L. monocytogenes* growth in vacuum packaged all-beef wieners. Rayman *et al.* (1981) reported that a combination of 3000 IU/g of nisin and 40 ppm of nitrite almost completely inhibited outgrowth of *Clostridium sporogenes* spores in meat slurries at 37 °C for 56 days. Pediocin BA28 from *P. acidilactici* BA28 was effective as a biopreservate for minced meat as 20,000 AU/ml are sufficient to check outbreaks of *Listeria*, Coliform, *Clostridia* and *Shigella* upto 30 days of incubation.

CONCLUSION

Pediococcus acidiactici BA28, a lactic acid bacteria, isolated from fecal sample of healthy individual, produces a bacteriocin (pediocin BA28) which is inhibitory to several human pathogens. LAB provides a high preservative

effect especially at low temperature at 4°C causing longer shelf life to the product over 21 days. It could also show that the potential of LAB to inhibit the growth of common food spoiling fungi opens up new perspectives for the bio-preservation of food products. This study concluded that the bacteriocin pediocin BA28 produced by *P. acidilactici* BA28 was demonstrated that inhibitory effects against bacterial pathogenic. Like nisin the bacteriocin also has the enormous potential for food applications as biopreservatives and probiotics.

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