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Research paper

Assessment of stability and biopreservative effect of recombinant pediocin CP2

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Abstract

The demand of GRAS food biopreservatives is rising day by day. and food industry is facing these challenges to provide naturally preserved foods with better consumer acceptability. Bacteriocins produced by lactic acid bacteria especially *Lactococcus lactis* and *Pediococcus acidilactici* offer the possibility of preventing food spoilage and improving counts of desirable bacteria in fermented foods. Pediocin CP2, a member of class IIa bacteriocin family, was engineered and expressed in *E. coli* BL21(DE3). Studies carried out to assess the stability and biopreservative effect of recombinant pediocin CP2 in some model food systems is reported. It was tested for biopreservation of spiked model food systems viz. a viz. black gram and mung bean sprouts, milk, minced meat, and vegetable fresh fruit cuts. Results indicated a gradual loss of pediocin activity with respect to time and nature of food material. Residual rec-pediocin alone exhibited a significant biopreservative effect ranging from 17 to 37% growth reduction of indicator organism in the absence of other antimicrobial factors and upto a maximum of 63% in combination with sodium citrate in the assayed time period. Results have clearly the potential of rec-pediocin in combination with chemical antimicrobial compounds in prolonging the shelf life of foods prone to microbial spoilage.

Keywords: *Pediococcus acidilactici*, pediocin CP2, recombinant pediocin, food biopreservatives, stability , pediocin.

Introduction

Lactic acid bacteria (LAB) have been traditionally used for fermenting food stuffs from the time immemorial. LAB metabolic activities significantly improve the nutritive value flavour, texture and acceptability of fermented foods (Schillinger and Lücke 1987) and also promote their microbial stability (Mensah *et al*., 1991). Antagonistic LAB play a vital role in the food preservation, which is manifested through the production of organic acids, CO_2, ethanol, H_2O_2 and diacetyl (Settanni and Corsetti, 2008), bacteriocins (De Vuyst and Vandamme, 1994), antifungal compounds such as phenyllactic acid (Corsetti *et al.*, 1998; Prema *et al*., 2008) and antibiotics such as reutericyclin (Höltzel *et al*., 2000).

Bacteriocins of LAB have great commercial potential as natural food preservatives due to their highly selective antimicrobial activities (Cotter *et al*., 2005). They are small, cationic, ribosomally synthesized, secretary peptides or proteins which may exhibit bactericidal or bacteriostatic effect on sensitive bacteria (Klaenhammer, 1988). Since the discovery of colicins by Gratia in 1925, bacteriocin production in starter culture of lactic acid bacteria (LAB) has attracted great interest in terms of food safety, due to their "generally recognised as safe" (GRAS) status. Currently, LAB bacteriocins enjoy the same status and this offer the food technologists the possibility of developing desirable flora in fermented foods or preventing food spoilage in both fermented and non-fermented foods by using broad- and narrow-spectrum bacteriocins, respectively (Settanni and Corsetti, 2008).

Pediocin CP2 is a member of class IIa bacteriocin family, purified and characterized from *Pediococcus acidilactici* MTCC 5101 (Kaur and Balgir, 2004; Kaur and Balgir, 2007; Kaur and Balgir, 2008; Balgir *et al.*, 2010). It possesses a wide antimicrobial spectrum against 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. From 2009 onwards, pediocin gene was engineered and its synthetic fusion gene construct was cloned and expressed using *E. coli* BL21(DE3)-pET32(b) system (Kumar *et al.*, 2012). Keeping in view the requirement of GRAS preservatives, this study was undertaken with the aim to explore stability and biopreservative potential of rec-pediocin in some model food systems.

Materials and Methods

Culture and growth conditions

E. coli BL21(DE3)-*pedA* was used for production of recombinant pediocin CP2. Strain was maintained on LB agar (Hi-media) containing 100µg/ml ampicillin. *Listeria monocytogenes* MTCC657 was used as an indicator/food spoiling agent in different model food systems. It was maintained on BHI agar (Hi-media).

Production and purification of recombinant pediocin CP2

Expression of recombinant pediocin was obtained in *E. coli* in BL21(DE3) using IPTG as an inducer. Rec-pediocin was expressed using T7 driven pET32(b)-*pedA* in periplasm as well as in the form of inclusion bodies (IBs). IBs were extracted from the cell lysates by urea lysis and rec-pediocin was renatured using refolding buffer containing 5mM immidazole and β-mercaptoethanol each. Since rec-pediocin protein bears two affinity purification tags, thus it was purified from crude cell extract by employing Ni-NTA affinity chromatography. Biological activity in rec-pediocin was induced after its processing with enterokinase enzyme that digested its N-terminal fragment bearing N-terminal affinity tags. Final purification step was based on streptactin affinity chromatography and highly pure rec-pediocin was obtained in the process. Active fractions were pooled and antimicrobial activity was assayed using standard bacteriocin assay (Kumar *et al.*, 2012).

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). Well diffusion assays were performed using 50µ of each dilution against *L. monocytogenes* MTCC 657 as reference microorganisms for the determination of a bacteriocin's biological activity.

Stability assay of rec-pediocin in model food systems

Rec-pediocin degradation was studied in different model food systems like pasteurized milk, minced meats, mung bean sprouts, black gram sprouts and vegetable fresh cuts obtained from various retail shops. 50g samples (in triplicates) were placed in air tight glass jars and 1000AU/ml pediocin solution was added to each and stored at 4ºC. Aliquots of samples were removed after every 48 h, centrifuged and supernatants assayed for pediocin activity.

Biopreservative effect of rec-pediocin in model food systems

50g fresh black gram and mung bean sprouts, minced meat and vegetable fresh cuts were taken in air tight glass jars and anti-listerial property of recombinant pediocin was studied at refrigeration temperature i.e. 4ºC for 7 days. Each test sample received a combination of pediocin (1000AU/ml) and other antimicrobial factors (100µg/ml) such as acetic acid, citric acid, EDTA, NaCl, sodium citrate, and sodium nitrite except for control. Samples were spiked with approx. 7 log units of *L. monocytogenes* and kept at 4^{o}C for 7 days. Listerial counts in treated and untreated spiked samples were observed at regular intervals by standard plate count technique. Finally, the percentage reduction in listerial counts was calculated using following formula proposed by Joshi *et al.*, (2006).

$$\text{\% Reduction of population} = \frac{\text{Reduction in microbial count}}{\text{Total count in control}} \times 100$$

Biopreservation of pasteurized milk

50ml pasteurized milk samples were pre-seeded with 10^7 cfu/ml *L. monocytogenes* using an overnight grown inoculum culture in BHI broth at 37ºC. Other milk preservatives (100µg/ml benzoic acid, salicylic acid, H_2O_2, boric acid, ammonium nitrate, and potassium nitrate) were also incorporated in the study to see their combined anti-listerial activity in presence of 1000AU/ml pediocin. No preservative was added to the control sample. Samples were incubated at 4ºC for 7 days. 100µl aliquots of each sample was withdrawn after every 24h and serially diluted and spread inoculated on BHI agar to observe listerial growth. Counts of indicator bacteria were recorded on a digital colony counter.

Statistical Analysis

Experimental data was expressed as mean value ± standard deviation. Statistical significance of the results were tested by one way ANOVA.

Results and Discussion

Stability of rec-pediocin

Stability of rec-pediocin was estimated over the entire assay time period in various model food systems (Table 1). Results indicated a gradual loss of its activity w.r.t. incubation time. In minced meat and vegetable fresh cuts, bacteriocin activity was lost within minutes of addition to 68 and 76% respectively. Recoverable pediocin activity fell to levels below 25% of the added to meat after 20 days when stored at 4ºC. Bacteriocin degradation followed a similar trend in all the refrigerated samples. In minced meat, pediocin deactivation was quite fast compared to others. Whereas, milk samples retained comparatively higher bacteriocin activity even on the 20th day of assay as compared to other samples.

Biopreservate effect of rec-pediocin in model food systems

Antilisterial combination consisting of 1000 AU/ml pediocin and 100µg/ml sodium nitrite significantly reduced number of pathogens by 4 to 5 log units in black gram and mung bean sprouts. An increase in total listerial counts from 7.5 to 9.35 log units was observed in control black gram sprouts stored at 4^{o}C after 7 days. Rec-pediocin alone reduced listerial growth by 17.93%. This anti-listerial effect further improved when antimicrobial properties of sodium citrate, sodium nitrite, acetic acid, NaCl, citric acid and EDTA were combined with it. Maximum growth lowering (4.55 log units) was reported in case of pediocin and sodium citrate combination where *L. monocytogenes* declined from 7.5 to 4.8 log cfu/ml, thereby causing 48.62% reduction compared to unpreserved control. Antilisterial activity remained constant upto 168 hours in the presence of other antimicrobials such as sodium citrate, sodium nitrite, NaCl and citric acid (Table 2).

Table 1: Stability of rec-pediocin in model food systems

Samples	% Residual activity of pediocin on					
	0 day	4 days	8 days	12 days	16 days	20 days
Pediocin	99.9 ± 0.1	99.9 ± 0.1	99.9 ± 0.1	99.9 ± 0.1	99 ± 0.1	98.5 ± 0.3
Black gram sprouts	84 ± 2	75 ± 4	66 ± 2	58 ±3	55 ±1	48 ±2
Mung bean sprouts	85 ± 1	76 ± 1	68 ± 3	59 ± 2	53 ±3	47 ±1
Milk	89 ± 1	82 ± 2	79 ± 1	73 ± 3	65 ± 1	53 ±1
Minced meat	68 ± 3	33 ± 2	32 ± 5	28 ±1	26 ±1	25 ±2
Vegetable fresh cuts	76 ± 1	67 ± 3	58 ± 3	51 ± 2	46 ±1	38 ±2

Mean + SD

Table 2: Preservative effect of rec-pediocin on black gram sprouts

Samples	log_{10} cfu/ml at								%growth
	0h	24h	48h	72h	96h	120h	144h	168h	reduction
Control	7.500 ± 0.021	7.612 ± 0.200	7.732 ± 0.127	8.013 ± 0.093	8.339 ± 0.004	8.611 ± 0.094	8.815 ± 0.073	9.346 ± 0.078	-
Pediocin	7.498 ± 0.153	7.203 ± 0.100	7.101 ± 0.109	6.859 ± 0.130	6.518 ± 0.137	6.490 ± 0.112	6.560 ± 0.105	7.670 ± 0.150	17.93
Acetic acid + Pediocin	7.473 ± 0.093	7.076 ± 0.069	6.577 ± 0.205	6.313 ± 0.128	5.818 ± 0.070	5.586 ± 0.020	5.321 ± 0.148	5.199 ± 0.076	44.38
Citric acid + Pediocin	7.458 ± 0.115	7.151 ± 0.035	6.836 ± 0.214	6.616 ± 0.108	6.082 ± 0.042	5.768 ± 0.059	5.510 ± 0.122	5.316 ± 0.130	43.12
EDTA + Pediocin	7.519 ± 0.180	6.939 ± 0.079	6.831 ± 0.115	6.681 ± 0.075	6.432 ± 0.087	6.309 ± 0.100	6.166 ± 0.166	5.898 ± 0.111	36.89
NaCl + Pediocin	7.412 ± 0.110	7.122 ± 0.040	6.827 ± 0.134	6.543 ± 0.083	6.101 ± 0.099	5.741 ± 0.045	5.493 ± 0.219	5.213 ± 0.100	44.22
Sodium citrate + Pediocin	7.499 ± 0.152	6.691 ± 0.056	6.218 ± 0.067	5.808 ± 0.014	5.518 ± 0.151	5.326 ± 0.120	5.121 ± 0.169	4.802 ± 0.072	48.62
Sodium nitrite + Pediocin	7.511 ± 0.130	6.811 ± 0.205	6.331 ± 0.160	6.013 ± 0.106	5.646 ± 0.131	5.485 ± 0.109	5.231 ± 0.113	5.018 ± 0.111	46.31

Mean ± standard Deviation

Spiked mung bean sprouts have 2.27 log units higher listeria under assayed conditions in absence of antimicrobial agents. In samples containing EDTA, sodium citrate, sodium nitrite and pediocin, 8.81, 10.93, 15.19 and 17.36% reduction in listerial counts were reported as compared to unpreserved control (Table 3). Antilisterial property enhanced further to 22.85, 23.36 and 53.58% levels upon addition of sodium citrate, EDTA and sodium nitrite respectively in combination with 1000AU/ml rec-pediocin. Maximum growth inhibition was reported in case of antimicrobial combination consisting of pediocin (1000AU/ml) and sodium nitrite (100µg/ml).

In case of freshly minced meat, samples that received pediocin and sodium citrate combination have 62.49% less microbial load as compared to unpreserved control, where log counts of *L. monocytogenes* increased from 7.48 to 10.68 within 168h at 4^{o}C. This combination has reduced initial inoculum load by 3.3 log units. It was followed by sodium nitrite (54.41% reduction) and acetic acid (53.84% reduction) in combination with pediocin (Table 4).

Pediocin alone exhibited 34.46% reduction in indicator growth in vegetable fresh cuts as compared to spiked unpreserved control where they multiplied from 7.56 to 9.86 log units. Sodium citrate and pediocin together significantly inhibited (55.49%) growth of indicator organism. As indicated in Table 5, samples receiving pediocin in combination with sodium nitrite, acetic acid, NaCl and citric acid have almost comparable reductions in listerial counts ranging from 43.82 to 50.62%.

Biopreservation of milk

Spiked treated and untreated milk samples indicated a difference of 2.5 to 5.5 listerial log units as given in Table 6. Pediocin's anti-listerial activity (25.91%) improved significantly when used in combination with ammonium nitrate (57.81%), potassium nitrate (55.71%), and benzoic acid (54.66%). Its combination with other standard milk preservatives such as boric acid (44.10%), salicylic acid (43.11%) and H_2O_2 (37.81%) was also effective in delaying outbreaks of *L. monocytogenes*. Maximum anti-listerial potency was observed in case of milk samples preserved using pediocin and ammonium nitrate, where log cfu/ml of *L. monocytogenes* reduced from 7.49 to 4.01 in the assay time period.

The health benefits of minimally processed and naturally preserved foods are becoming more and more attractive. In the last decades, food industry is facing conflicting challenges to replace chemical preservatives with GRAS biopreservatives and provide traditionally fermented foods to health conscious consumers. In lieu of that, FDA has approved for incorporation of nisin (produced by *Lactococcus lactis*) as a bio-preservative in many foods (Federal Register, 1988). It has very high potency in dairy products, but apparently it is not the bacteriocin of choice for preserving meat and meat products. Bacteriocins produced by *Pediococcus, Leuconostoc, Caynobacterium* and *Lactobacillus* sp., isolated from naturally fermented meat products are likely to have much greater potential as meat preservative (Stiles and Hastings, 1991).

Table 3: Preservative effect of rec-pediocin on spiked mung bean sprouts

Samples	log_{10} cfu/ml at								% growth
	0h	24h	48h	72h	96h	120h	144h	168h	reduction
Control	7.569 ± 0.093	7.721 ± 0.188	7.943 ± 0.101	8.321 ± 0.226	8.764 ± 0.095	9.198 ± 0.080	9.511 ± 0.041	9.839 ± 0.113	-
Pediocin	7.612 ± 0.106	7.689 ± 0.088	7.738 ± 0.159	7.843 ± 0.201	7.901 ± 0.137	7.984 ± 0.110	8.001 ± 0.127	8.131 ± 0.108	17.36
EDTA	7.557 ± 0.110	7.721 ± 0.231	7.938 ± 0.093	8.146 ± 0.169	8.359 ± 0.133	8.563 ± 0.099	8.771 ± 0.150	8.972 ± 0.145	8.81
EDTA + Pediocin	7.523 ± 0.109	7.528 ± 0.168	7.530 ± 0.110	7.533 ± 0.107	7.536 ± 0.158	7.537 ± 0.056	7.539 ± 0.119	7.540 ± 0.107	23.36
Sodium citrate	7.572 ± 0.091	7.621 ± 0.200	7.854 ± 0.108	8.017 ± 0.168	8.213 ± 0.291	8.426 ± 0.103	8.569 ± 0.088	8.763 ± 0.101	10.93
Sodium citrate + Pediocin	7.528 ± 0.115	7.538 ± 0.104	7.543 ± 0.101	7.549 ± 0.068	7.562 ± 0.101	7.579 ± 0.147	7.583 ± 0.098	7.591 ± 0.074	22.85
Sodium nitrite	7.561 ± 0.081	7.623 ± 0.124	7.754 ± 0.112	7.871 ± 0.227	7.913 ± 0.094	8.109 ± 0.189	8.212 ± 0.118	8.344 ± 0.122	15.19
Sodium nitrite + Pediocin	7.439 ± 0.099	6.613 ± 0.077	5.826 ± 0.095	5.237 ± 0.209	4.816 ± 0.096	4.725 ± 0.077	4.638 ± 0.104	4.567 ± 0.115	53.58

Mean ± standard Deviation

Table 4: Preservative effect of rec-pediocin in minced meat

Samples	log_{10} cfu/ml at								% growth reduction
	0h	24h	48h	72h	96h	120h	144h	168h	
Control	7.478 ± 0.084	7.898 ± 0.078	8.217 ± 0.114	8.743 ± 0.079	9.114 ± 0.270	9.674 ± 0.126	10.101 ± 0.113	10.678 ± 0.089	-
Pediocin	7.493 ± 0.223	7.330 ± 0.127	7.220 ± 0.166	7.126 ± 0.109	7.006 ± 0.099	6.924 ± 0.168	6.816 ± 0.127	6.762 ± 0.106	36.67
Acetic acid + Pediocin	7.464 ± 0.174	7.219 ± 0.118	6.999 ± 0.107	6.641 ± 0.808	6.118 ± 0.159	5.774 ± 0.129	5.210 ± 0.128	4.929 ± 0.097	53.84
Citric acid + Pediocin	7.512 ± 0.116	7.456 ± 0.110	7.189 ± 0.073	6.821 ± 0.097	6.543 ± 0.266	6.174 ± 0.102	5.899 ± 0.116	5.463 ± 0.116	48.54
EDTA + Pediocin	7.488 ± 0.122	7.386 ± 0.091	7.297 ± 0.130	7.148 ± 0.098	7.098 ± 0.185	6.979 ± 0.122	6.896 ± 0.110	6.794 ± 0.089	36.37
NaCl + Pediocin	7.508 ± 0.101	7.421 ± 0.129	7.091 ± 0.067	6.763 ± 0.106	6.418 ± 0.223	5.922 ± 0.080	5.489 ± 0.137	5.296 ± 0.094	50.40
Sodium citrate + Pediocin	7.321 ± 0.118	6.990 ± 0.156	6.580 ± 0.049	6.019 ± 0.110	5.519 ± 0.213	5.023 ± 0.132	4.510 ± 0.318	4.005 ± 0.134	62.49
Sodium citrate + Pediocin	7.413 ± 0.095	7.121 ± 0.106	6.917 ± 0.119	6.523 ± 0.091	6.043 ± 0.217	5.618 ± 0.079	5.116 ± 0.136	4.761 ± 0.089	54.41

Mean ± standard Deviation

Table 5: Preservative effect of rec-pediocin on vegetable fresh cuts

Samples	log_{10} cfu/ml at								% growth reduction
	0h	24h	48h	72h	96h	120h	144h	168h	
Control	7.571 ± 0.067	7.632 ± 0.112	7.858 ± 0.112	8.223 ± 0.105	8.639 ± 0.114	8.986 ± 0.108	9.215 ± 0.121	9.856 ± 0.122	-
Pediocin	7.507 ± 0.136	7.230 ± 0.107	7.197 ± 0.146	7.041 ± 0.068	6.908 ± 0.074	6.779 ± 0.111	6.616 ± 0.087	6.459 ± 0.103	34.46
Acetic acid + Pediocin	7.543 ± 0.118	7.176 ± 0.014	6.780 ± 0.164	6.213 ± 0.109	5.810 ± 0.076	5.576 ± 0.097	5.232 ± 0.111	5.001 ± 0.181	49.26
Citric acid + Pediocin	7.419 ± 0.020	6.988 ± 0.105	6.731 ± 0.134	6.587 ± 0.112	6.332 ± 0.116	6.009 ± 0.164	5.168 ± 0.074	5.537 ± 0.121	43.82
EDTA + Pediocin	7.548 ± 0.166	7.289 ± 0.171	7.217 ± 0.105	7.157 ± 0.088	6.949 ± 0.090	6.824 ± 0.116	6.710 ± 0.141	6.467 ± 0.094	34.38
NaCl + Pediocin	7.558 ± 0.202	7.298 ± 0.141	6.876 ± 0.112	6.327 ± 0.111	5.901 ± 0.060	5.600 ± 0.113	5.381 ± 0.103	5.126 ± 0.023	47.99
Sodium citrate + Pediocin	7.478 ± 0.150	6.713 ± 0.117	6.280 ± 0.135	5.772 ± 0.107	5.236 ± 0.110	4.993 ± 0.108	4.621 ± 0.125	4.387 ± 0.098	55.49
Sodium nitrite + Pediocin	7.510 ± 0.183	6.782 ± 0.170	6.278 ± 0.150	5.800 ± 0.117	5.411 ± 0.073	5.186 ± 0.076	5.020 ± 0.159	4.867 ± 0.101	50.62

Mean ± standard Deviation

Table 6: Preservative effect of rec-pediocin on milk

Samples	log_{10} cfu/ml at								% growth reduction
	0h	24h	48h	72h	96h	120h	144h	168h	
Control	7.566 ± 0.050	7.621 ± 0.179	7.718 ± 0.150	7.913 ± 0.120	8,149 ± 0.165	8.346 ± 0.135	8.515 ± 0.215	9.510 ± 0.145	-
Pediocin	7.497 ± 0.169	7.105 ± 0.142	6.931 ± 0.190	6.708 ± 0.241	6.518 ± 0.121	6.609 ± 0.175	6.760 ± 0.166	7.046 ± 0.137	25.91
Benzoic acid + Pediocin	7.413 ± 0.134	6.976 ± 0.560	6.671 ± 0.166	6.213 ± 0.312	5.760 ± 0.157	5.299 ± 0.111	4.811 ± 0.180	4.312 ± 0.166	54.66
Salicylic acid+ Pediocin	7.518 ± 0.109	7.251 ± 0.161	6.936 ± 0.248	6,717 ± 0.190	6.310 ± 0.267	6.068 ± 0.185	5.721 ± 0.316	5.419 ± 0.165	43.11
H_2O_2+ Pediocin	7.519 ± 0.182	6. 989 ± 0.335	6.848 ± 0.17	6.687 ± 0.211	6.479 ± 0.160	6.419 ± 0.114	6.116 ± 0.080	5.914 ± 0.209	37.81
Boric acid+ Pediocin	7.416 ± 0.114	7.208 ± 0.067	6.836 ± 0.115	6.613 ± 0.222	6.282 ± 0.125	5.981 ± 0.550	5.668 ± 0.120	5.317 ± 0.210	44.10
Ammonium nitrate + Pediocin	7.488 ± 0.194	6.891 ± 0.242	6.473 ± 0.204	6.007 ± 0.079	5.581 ± 0.210	5.003 ± 0.151	4.685 ± 0.095	4.012 ± 0.172	57.81
Potassium nitrate + Pediocin	7.349 ± 0.124	6.911 ± 0.591	6.531 ± 0.210	6.103 ± 0.230	5.616 ± 0.114	5.017 ± 0.171	4.793 ± 0.140	4.212 ± 0.144	55.71

Mean ± standard Deviation

L. monocytogenes has been associated with many food products such as raw milk, processed milk, chesses, ice creams, raw vegetables, fresh raw meat sausages, raw and cooked poultry, fish and meat of all types. It has ability to multiply at refrigeration temperature and under anaerobic conditions. US government has set zero tolerance levels for *L. monocytogenes* in ready to eat meats (Varma *et al*., 2007). Several physical and chemical treatments are used in food industry to overcome listerial spoilage of foods (Beuchat, 1995; Ukuku and Fett, 2004; Bari *et al*., 2005; Molinos *et al*., 2005). Most of these studies reported enhanced antilisterial activity of bacteriocins in presence of other antimicrobial agents such as organic acids and their salts (e.g. acetic acid, citric acid, phytic acid, potassium sorbate, sodium lactate and sodium propionate), chelating agents and disinfectants. Similar observations are recorded in this study, as antilisterial activity of rec-pediocin has improved significantly (p-value < 0.05) upon inclusion of organic acids and their salts, NaCl and EDTA. This might be due to induction of conformational changes in bacteriocin molecules or due to changes in membrane permeability of the target organism in presence of NaCl (Lee *et. al*., 1993; Jydegaard *et al*., 2000). Organic acids and their salts are known to potentiate antimicrobial activity of pediocins greatly, as acidification causes an increase in net positive charge on the molecules (Stiles, 1996; Scannell *et al*., 1997; Ukuku and Fett, 2004) and facilitates their diffusion through cell walls as interactions of bacteriocin molecules with cell membranes of sensitive bacteria are purely electrostatic in nature (Montville and Chen, 1998). Chelating agents permeate outer membrane of indicator bacteria by extracting calcium and magnesium ions that stabilize lipopolysaccharide structure and allowing bacteriocins to reach cytoplasmic membranes (Vaara, 1992; Schved *et al*., 1994; Helander *et al*., 1997).

Results also indicated a drop in recoverable pediocin activity which can be ascribed to binding of the bacteriocin with meat proteins or fats or due to abnormal proteolytic digestion (Mills *et al*., 2011). In case of minced meat, sprouts and vegetable fresh cuts, particle size was thought to control degradation of rec-pediocin and its antimicrobial activity against *L. monocytogenes* as reported previously by Dickson and others (Iowa State University, USA.). Although rec-pediocin showed a great promise as food preservative, further studies are considered necessary in the area of mechanism of bacteriocin action on other food borne and food spoilage organisms. A concentration dependent shelf life analysis of recombinant pediocin in various food systems is desirable, before system can be developed that fully uses its potential.

With the increasing demand for minimally processed foods, bacteriocins are providing ample opportunity for their widespread food applications. Moreover, growing consumer refusal of chemical additives to combat food spoilage, there is increasing demand for these biopreservatives with prolonged antimicrobial effect and a highly specific antimicrobial spectrum. Bacteriocins such as nisin, pediocin PA-1, enterocin AS-48, and lacticin 3147, have been reported to exhibit a large spectrum of food applications. But, due to some legal restrictions, nisin is the only FDA approved bacteriocin exploited in food industries of about 50 countries. Genetic manipulation approaches are being tested to widen antimicrobial spectrum and stability of bacterioicins and their heterologous expression in strains of industrial importance (Halami and Chandrashekhar, 2007; Tominaga and Hatakeyama, 2007). Engineered bacteriocins with added features could be very rewarding from a food safety and economic perspective point is view. But at the same time, there is a need to address consumer concerns regarding food safety too. Continued research will lead to an increased understanding and application of these new food biopreservatives.

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