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Growth control of *listeria monocytogenes* in experimental cheese samples by *lactobacillus casei* RN 78 and its bacteriocin

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Anti-listerial effect of freeze dried fractions of partially purified bacteriocin (Fd-PPL) and the producer strain *Lactobacillus casei* RN 78 strain was evaluated in experimental cheese samples during storage at two different temperatures (4 and 35˚C). With the addition of 6400 AU/g of Fd- PPL fractions to the cheese samples of the initial concentration of 4.81 +/- 0.06 log CFU/ml of *Listeria monocytogenes* was reduced up to 0.91 +/- 0.01 log CFU/ml. Whilst, in the presence of 10^7 CFU/ml live bacterial cells of *L. casei* RN 78 of the viability of the sensitive cells decreased sharply (0.71 log CFU/ml). The effect was more pronounced after 24 h of incubation and high levels of antibacterial activity (12800 AU/ml) was seen in these samples. An enhanced reduction in the pH was recorded (4.8 - 5.0) in the cheese samples inoculated with live producer cells compared to control samples without any enrichment. A synergistic bactericidal effect of Lactocin RN 78 in combination with 3% Sodium chloride in cheese samples was observed, and *L. monocytogenes* population were reduced to 0.69 log CFU/ml within 90 days of incubation, at 35˚C. In contrast to the producer strain, the antibacterial effect of Lactocin was more pronounced in cheese samples stored at 4˚C. The texture of the experimental cheese samples including odour, colour and consistency in different batches were also recorded through out the study.

Key words: *Lactobacillus casei*, anti-listerial, bacteriocin, cheese, biological preservative.

INTRODUCTION

Listeria monocytogenes, the bacteria responsible for causing listeriosis is ubiquitous in the environment and is resistant to low temperatures, low pH and high salt concentrations (Adams and Moss, 1995). In the last decade, out breaks of listeriosis associated with cheese consumption were of great concern to the dairy industry because of the number of cases and the overall mortality rate reported (Rudolf and Scherer, 2001; Sanchez-Rey et al., 1993). Greater attention is being drawn towards application of Lactic acid bacteria (LAB) which are considered as natural and safe, and its food preservation action attributed to bacteriocins. Bacteriocins are biologically active peptides or protein complexes that display a bactericidal mode of action almost exclusively toward closely related species (Tagg et al., 1976). Different bacterial species of LAB are bacteriocin producers and are found active against food spoilage and food borne pathogenic microorganisms including *Bacillus cereus, Clostridium perfringens, Staphylococcus aureus*, and *L. monocytogenes* (Chen and Hoover, 2003). Among bac-teriocins so far characterized, nisin is the best defined, and the only purified bacteriocin produced by *Lactococcus lactis*, that has been approved for use in food products in almost 45 countries (Delves-Broughton, 1990; Cleveland et al., 2001

Beside, nisin and pediocin has also been approved for use in food products. Moreover, bacteriocins are innocuous due to proteolytic degradation in the gastrointestinal (GI) tract, and are often found over

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often found stable over several months during frozen and refrigeration storage and after drying (Ryan et al., 1998). Certain cheeses may be at risk of contamination with pathogenic bacteria such as *Listeria*. Additionally, the growth of Listeria at refrigerated temperature poses great threat to foods especially dairy products. Several researchers have tried to use anti-listerial bacteriocin producing strains as a means of protection in various cheeses. Some of the successful attempts made were in the cases of Cheddar cheese (Leroy et al., 2003; Moreno et al., 2003), Manchego cheese (Nunez et al., 1997; Garcia et al., 1997), Talegio cheese (Giraffa and Carminati, 1997) and goats milk cheese (Farias et al., 1999). These natural metabolites could replace the use of chemical additives such as sorbic acid, sulfur dioxide, nitrite, nitrate, and others are used as bio-preservatives. In the present study, the possibility of developing a bio-preservative was investigated by determining the anti-listeria effect of a bacteriocin producing *Lactobacillus casei* RN78 in experimental cheese samples during storage at 4 and 35˚C.

MATERIALS AND METHODS

Bacterial strains, media and culture conditions

A previously isolated anti-listeria bacteriocin producer *L. casei RN 78* (Mojgani and Amirinia, 2007; Mojgani and Esmailkhanian, 2006; Mojgani et al., 2006) used as producer strain was grown in DeMan Rogosa and Sharpe broth MRS (Merck, Germany) under anaerobic conditions at 35˚C for 24 - 48 h. *L. monocytogenes* ATCC 1315 used for challenge experiment was grown aerobically in Trypticase Soya Broth (Merck, Germany) and Brain Heart Infusion (BHI, Himedia, India) at 37˚C for 24 h. The strains were maintained as master seed in a frozen stock with 15% glycerol at –20˚C. Working seed was prepared by sub-culturing in the master seed twice in skim milk supplemented with 0.3% yeast extract at 35˚C for 24 h.

Preparation of freeze dried-partially purified bacteriocin fractions

Lactocin RN 78 was partially purified by ammonium sulfate precipitation at final concentrations of 80% w/v (Bollag and Edelstein, 1991). Initially, 2 liter culture of the producer strain was grown in MRS broth with 3% yeast extract at 37˚C for 48 h. The supernatant was then collected via centrifugation at 3000 rpm, 20 min, 4˚C and pH adjusted to 6.5 by 1N NaOH. The collected neutralized supernatant fluids were concentrated to one tenth of its original volumes by vacuum drying, and then subjected to four rounds of 20% ammonium sulphate precipitation. The precipitate at the final round was recovered by centrifugation (11,200 × *g*, 15 min, 4˚C), resuspended in sterile MilliQ water and dialyzed against sterile MilliQ water at 8˚C for 24 h, using Sephadex G-25 (coarse) column (2 × 5 cm; Bio- Rad) equilibrated with 10 mM phosphate buffer pH 7. The pH of the desalted sample (PPL) was adjusted between 6.5 and 7.0 with 0.1 N NaOH and frozen at -70˚C for 48 h and freeze dried by vacuum evaporation to obtain dry powder form (Fd-PPL) . Main drying cycle was performed at 15˚C for 15 h at a pressure of 22 Pa. The minimum inhibitory concentration of the Fd-PPL fractions against *L. monocytogenes* was evaluated by critical dilution assay described by Mayr-Hartings and colleagues (1972). One AU (arbitrary unit) was defined as reciprocal of the highest serial of two fold dilution showing inhibition of the indicator strain and the activity recorded in AU/ml.

Preparation of cheese samples

Cheese was manufactured from pasteurized (72˚C for 15 s) cow milk. The milk at 39˚C was distributed into four 12-liter vats. Commercial starter cultures were inoculated in all milk vats at 2% inoculation level and mixed well till the temperature reaches 35˚C. Rennet (0.015 g/liter, Maxiren 150; Gist- brocades, Delft, The Netherlands) was added to milk 20 min after inoculation. The curds were cut at 45 min after rennet addition, into 6 - 8-mm cube. Whey was drained off, and curds were distributed into plastic cylindrical molds. Six cheeses (approximately 200 g in weight) were obtained from each vat making a total of 24 cheese samples. Finally, all the samples were packaged in Cryovac plastic bags under sterile condi-tions and stored at refrigerated temperature until use. All materials and equipment were autoclaved before and after cheese making.

In-vivo experiments

The cheese samples were distributed into two groups of 12 samples each. The cheese samples were added with *L. casei* RN 78 viable cells (L), Lactocin RN 78 (B), NaCl (N) and *L. monocytogenes* (P) after preparation as follows:

a) Preparation of producer cells: The live bacterial cells (10^7 cfu/ml) of *L. casei* RN78 were prepared in 5 ml normal saline solution and the mixture poured on cheese samples and shaken vigorously for approximately 2 min,
b) Preparation of *L. monocytogenes:* A 16 h old culture of the said pathogen was diluted in 5 ml normal saline to achieve 10^4 cfu/ml bacterial counts. The mixture was applied to cheese samples as described above, c) Preparation of Fd-PPL: The Freeze dried partially purified fractions of lactocin RN78 in dry powder forms were dissolved in 5 ml PBS buffer (pH 5.5) to achieve 6400 AU/ml of activity. The diluted mixture was applied to cheese samples as described above. Table 1 shows the cheese samples prepared with different enrichments. A group of cheese samples were stored at 4˚C and the other at 37˚C. All samples were analyzed for bacterial load (cfu/ml), antibacterial activity (AU/ml), pH, and cheese texture on days 3, 7, 15, 30, 60 and 90.

Enumeration of *L. monocytogenes*

The number of viable *L. monocytogenes* organisms in cheese samples was determined during mentioned time periods. A cube (6.0 by 6.0 by 0.5 cm) was randomly cut from each sides of cheese block with a sterile kitchen knife, and in addition, a core sample (2.0 by 2.0 by 3.0 cm) was removed from the interior of the cheese block. The cheese cubes and core (total weight, approximately 200 g) were placed into a stomacher bag and minced with a sterile knife.

The resulting pieces were mixed thoroughly, and then a 10-g portion was removed and placed into another stomacher bag and macerated. Serial dilutions of the homogenized mixture in 0.1% peptone water were spread plated onto Listeria enrichment (Hi-Media) agar and incubated at 37˚C for 48 h, before enumeration of *L. monocytogenes* colonies. The absorbance (O.D) at 660 nm and viable count (cfu/ml) was determined as described earlier.

Bacteriocin assay

Cheese samples (5 g) were homogenized with 10 ml of sterile 0.02

Table 1. Preparation of cheese samples with different enrichments.

No	Cheese samples	*L. casei* RN78 (L)	Fd-PPL fractions (B)	NaCl (N)	*L. monocytogenes* (P)
1	LP	10^7 cfu/ml	-	-	10^4 cfu/ml
2	BP	-	6400 AU/ml	-	10^4 cfu/ml
3	NP	-	-	3%	10^4 cfu/ml
4	LN	10^7 cfu/ml	-	3%	
5	LBP	10^7 cfu/ml	6400 AU/ml	-	10^4 cfu/ml
6	BNP	-	6400 AU/ml	3%	10^4 cfu/ml
7	LNP	10^7 cfu/ml	-	3%	10^4 cfu/ml
8	L	10^7 cfu/ml	-	-	
9	P	-	-	-	10^4 cfu/ml
10	B	-	6400 AU/ml	-	-
11	N	-	-	3%	-
12	O	-	-	-	-

N HCl at 50˚C and centrifuged (12,000 x *g*, 20 min, 4˚C) and the supernatant was collected. Neutralized supernatants were placed in triplicate into wells (5-mm diameter) made in pour plates of BHI agar inoculated with 1% of a 16-h culture of *L. monocytogenes.* After allowing pre- diffusion at 4˚C, 2 h the plates were incubated at 37˚C for 48 h. The titer of the bacteriocin activity was quantified and expressed in arbitrary units (AU/ml).

Determination of cheese pH

Cheese pH was measured in duplicate after mentioned time intervals with a penetration electrode (model 52 - 3, 2; Crison Instruments S.A., Barcelona, Spain) by means of a Crison GPL 22 pH meter.

Determination of cheese texture

All cheese samples were analyzed at different time intervals for their texture including colour, consistency, odour and overall appearance and graded accordingly. The cheese samples were assessed by a commercial grader from a local cheese factory.

RESULTS

A bacteriocin (Lactocin RN78) producing *L. casei* RN78 strain isolated from a cheese sample in our previous studies was further evaluated for *in vivo* efficacy in control of *L. monocytogenes*. Different combinations of cheese samples with 6400 AU/ml Lactocin RN78 (B), 10^7 CFU/ml producer strain *L. casei* RN78 (L), and 3% NaCl (N) were challenged with 10^4 CFU/ml *L. monocytogenes* (P) during storage at two different temperatures (4 and 35˚C). Initially, the culture supernatant fluid of the producer strain containing lactocin RN78 were partially purified and freeze dried. These freeze dried partially purified preparations (Fd-PPL) were applied to cheese samples and evaluated. During the purification procedures, each step resulted in a considerable loss of protein concentration while specific activity increased (4970 AU/ml). The optimal bacteriocin recovery was achieved by 80% ammonium sulphate saturation. During first week of storage at both the temperatures, the count of *L. monocytogenes* decreased in all the samples by approximately 1.0 - 2.0 log cfu units, respectively, compared with initial levels of contamination. The producer strain *L. casei* RN78 was able to grow in cheese samples and reached a final concentration of 10.1 +/- 0.01 log CFU per/ml within 24 - 48 h of incuba-tion at 35˚C. Simultaneously, with the increase in growth of the producer cells a substantial amount of antibacterial activity (12800 AU/ml) and a reduction in Listeria count was detected.

As Table 2 depicts, the cell count of *L. monocytogenes* increased initially during the first week of incubation and later a decrease was recorded in almost all the samples. Figure 1 illustrates the rapid cell death of the sensitive cells in the presence of viable *L. casei* RN78 cells (LP), compared to the untreated samples (P). Highest reduce-tion in the viability of this pathogen was recorded in these samples during storage at 35˚C, and 2 - 3 log units' reduction of the pathogen was seen. The samples containing viable producer cells were more effective in listeria growth inhibition during storage at 35˚C, while their growth was negligible within 3 days of storage at 4˚C. With decrease in the viability of the producer strain, the number of sensitive cells increased and reached to its maximum within 3 - 5 days of incubation. Consistent with their growth antibacterial activity was also recorded.

According to Table 3, addition of 6400 AU/ml of Fd-PPL correlated with a 1,000-fold reduction in *L. monocytogenes* numbers in these cheese samples. The bacteriocin containing samples (BP and BNP) were more effective and higher bactericidal activity (AU/ml) was recorded in samples stored at refrigerated temperature. In contrast, the samples inoculated with live producer cells (LP, LBP, LNP), showed higher bactericidal activity when stored at 35˚C. Reduction was enhanced in the presence of bacteriocin (BP) and NaCl (NP) containing

Table 2. Survivors of *L. monocytogenes* (log CFU/ml) in experimental cheese samples stored at two different temperatures.

Cheese samples	4˚C					35˚C				
	Days					Days				
	3	15	30	60	90	3	15	30	60	90
LP	5.56	5.50	5.41	5.31	5.30	5.88	2.94	1.66	1.01	0.99
BP	5.81	3.16	2.06	0.90	0.86	5.81	3.08	2.10	0.97	0.91
NP	5.40	2.99	3.00	2.00	2.90	5.31	4.99	3.32	3.70	3.78
NL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BNP	5.48	1.46	0.4	0.01	0.0	5.42	1.26	1.91	0.87	0.79
LBP	5.09	2.45	2.02	1.01	0.71	5.82	1.09	0.0	0.0	0.0
LNP	5.86	3.65	3.59	3.08	3.00	5.78	1.97	1.71	1.26	1.11
L	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P	5.76	5.67	5.56	5.51	5.55	5.86	6.00	5.69	5.17	5.19
B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Cheese samples containing LP: *L. casei* RN 78 and *L. monocytogenes,* BP: Lactocin RN78 and *L. monocytogenes*, NP: NaCl and *L. monocytogenes*, NL: NaCl and *L. casei* RN78, BNP: Lactocin RN78, NaCl and *L. monocytogenes*, LBP: *L. casei,* Lactocin RN78 and *L. monocytogenes,* L: *L. casei* RN78, P: *L. monocytogenes,* B: Lactocin RN78, N: NaCl. ND: Not determined.

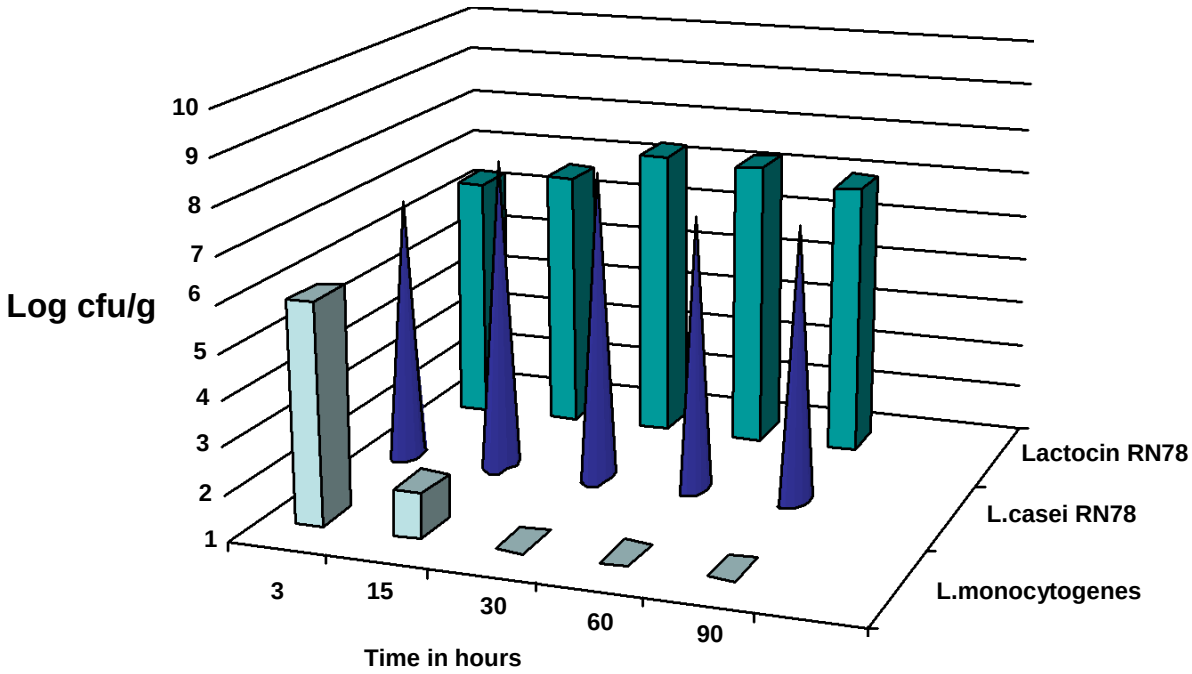

Figure 1. Lactocin RN78 activity (AU/ml) and growth rate (Log cfu/ml) of *L. casei* RN78 and *L. monocytogenes* in experimental cheese samples stored at 35˚C.

cheese samples at 4˚C by almost 2.51 and 1.77 Log cycles, respectively. The level of survival of *L. monocytogenes*, in the presence of cheese samples with producer cells in combination with the bacteriocin (LBP) and NaCl (LNP), and samples with the bacteriocin and NaCl (BNP), decreased considerably within a month of storage at both temperatures. In combination, NaCl and bacteriocin were able to completely inhibit the growth of the said pathogen at 4˚C, while at 35˚C 0.79 log cfu/ml of the challenge strain were viable even after 90 days of storage. The anti-listerial effect of NaCl alone was significant only during initial weeks of storage and then

Table 3. Lactocin RN78 activity (AU/ml) in experimental cheese samples stored at two different temperatures.

Cheese samples	4ºC					35ºC				
	Days					Days				
	3	15	30	60	90	3	15	30	60	90
LP	800	3200	3200	800	800	6400	6400	6400	1600	1600
BP	6400	6400	1600	1600	1600	3200	1600	1600	800	800
NP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
NL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BNP	3200	1600	1600	800	800	1600	1600	1600	1600	1600
LBP	6400	3200	3200	3200	3200	12800	12800	12800	12800	6400
LNP	3200	3200	800	800	800	6400	12800	12800	3200	3200
L	12800	12800	12800	6400	6400	6400	12800	12800	6400	3200
P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B	6400	6400	3200	3200	3200	6400	6400	6400	3200	3200
N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Cheese samples containing LP: *L. casei* RN 78 and *L. monocytogenes,* BP: Lactocin RN78 and *L. monocytogenes*, NP: NaCl and *L. monocytogenes*, NL: NaCl and *L. casei* RN78, BNP: Lactocin RN78, NaCl and *L. monocytogenes*, LBP: *L. casei,* Lactocin RN78 and *L. monocytogenes,* L: *L. casei* RN78, P: *L. monocytogenes,* B: Lactocin RN78, N: NaCl. ND: Not determined.

Table 4. Values of pH in experimental cheese samples stored at two different temperatures.

Cheese samples	4ºC					35ºC				
	Days					Days				
	3	15	30	60	90	3	15	30	60	90
LP	5.94	5.76	5.77	5.70	5.70	5.41	5.18	5.00	5.00	4.77
BP	5.54	5.60	5.62	5.61	5.66	5.54	5.60	5.71	5.77	5.79
NP	5.82	5.94	5.91	5.90	5.90	5.60	5.56	5.98	5.99	5.99
NL	5.79	5.99	5.98	5.99	5.99	5.55	5.45	5.41	5.40	5.50
BNP	5.57	5.67	5.69	5.84	5.84	5.59	6.20	6.91	6.90	6.99
LBP	5.59	5.57	5.57	5.56	5.51	5.52	5.63	5.60	5.00	5.01
LNP	5.91	6.09	6.00	5.99	6.00	5.55	5.50	5.47	5.30	5.54
L	5.90	5.76	5.57	5.57	5.50	5.41	5.18	5.00	5.00	4.98
P	5.54	5.40	5.52	5.50	5.60	5.54	5.40	5.52	5.50	5.60
B	5.68	5.91	5.93	6.47	6.41	5.90	5.96	5.94	5.94	5.99
N	5.85	5.99	6.11	6.10	6.00	5.85	5.99	6.11	6.10	6.00
O	5.59	5.61	5.60	5.60	5.58	5.57	5.54	5.64	5.41	5.40

Cheese samples containing LP: *L. casei* RN 78 and *L. monocytogenes,* BP: Lactocin RN78 and *L. monocytogenes*, NP: NaCl and *L. monocytogenes*, NL: NaCl and *L. casei* RN78, BNP: Lactocin RN78, NaCl and *L. monocytogenes*, LBP: *L. casei* RN78*,* Lactocin RN78 and *L. monocytogenes,* L: *L. casei* RN78, P: *L. monocytogenes,* B: Lactocin RN78, N: NaCl, O: untreated cheese sample.

after remained, almost constant and no significant reduction of listeria growth was recorded in these samples (NP). Compared to the effect of NaCl, the bacteriocin (Fd-PPL) fractions appeared more efficient in reducing the growth of *L. monocytogen* in these cheese samples and by the end of 90 days only 0.8 - 0.9 log/cfu/ml of the pathogen was able to survive in these samples. Table 4 illustrates the evolution of pH in the cheese samples during storage at both temperatures. An increase in pH in all the samples except for those inoculated with bacteriocinogenic strain was recorded after almost a day of incubation at both the temperatures. The alteration in pH was more evident mainly in samples containing NaCl (NP and BNP). The untreated cheese

sample (O) also showed an increase in pH after almost a week of incubation at room temperature and after a month of storage at refrigerated temperature. In contrast, the cheese pH was decreased significantly ($p < 0.001$) in the samples containing the viable producer cells. In these samples, the values of pH was between 4.8 and 5.2 on day 3 which reached 4.6 - 4.9 by the end of 7 weeks after almost a week of storage at 35°C. The reduction in the pH appeared is related to an increase in antibacterial activity and reciprocally a higher killing rate of *L. monocytogenes*.

The texture of the cheeses including odours, and appearance appeared affected in the treated and untreated cheese samples during storage at room temperature. Spoilage was marked as development of unpleasant odour, discolouration and growth of moulds on the surface. Samples stored at room temperature spoiled much earlier than the samples stored at refrigerated temperature, except for bacteriocin treated samples which showed no spoilage during the test period while storage at room temperature. No such effect was recorded in samples stored at refrigerated temperatures. The untreated samples showed discolouration and putrid aroma after only two days at room temperature and after a week of incubation at refrigerated temperature.

DISCUSSION

In our previous studies we have reported a Lactocin producing strain *L. casei* RN78 isolated from a local cheese sample (Mojgani et al., 2006; Mojgani and Esmailkhanian, 2006; Mojgani and Amirinia, 2007). In *vitro* characterization of Lactocin RN78 showed qualities which made it an interesting candidate for application in food system. In this study, to exploit the insitu anti-listerial efficacy of the mentioned bacteriocinogenic strain, cheese samples enriched with 10^7 cfu/ml live producer cells, 6400 AU/ml bacteriocin and 3% NaCl were pre-pared and challenged with 10^4 cfu/ml *L. monocytogenes* cells. Throughout the studies, freeze dried preparations of lactocin RN78 (Fd- PPL) were used as previous reports had shown instability of liquid preparations of bacteriocin due to their high water content. The stability of Fd-PPL fractions similar to that observed previously for lacticin 3147 and nisin, (Delves -Brroughton et al., 1996; Kanatani et al., 1992; Kawai et al., 1997). The advantages and importance of addition of bacteriocinogenic strain to the cheese has been reported previously. Concurrently, the *L. casei* RN78 cells were able to grow and exert anti-listerial activity in these cheese samples mainly during storage at 35°C. High growth rate of bacteriocinogenic strain co-related with high bactericidal activity and high growth inhibiting rate of Listeria. In situ production of the bacteriocin by *L. casei* RN78 appeared to be responsible for the appreciable decline in the number of *L. monocytogenes* cells in the cheese samples, a phenolmenon similar to the effect of pediocin produced by strain MM217 (Buyong et al.,1998; Rodriguez et al., 2005). Similar to our *in vitro* results, Lactocin RN78 acted synergistically with 3% NaCl in BNP cheese samples and enhanced antibacterial activity against *L. monocytogenes* was seen in these samples compared to samples without the salt. These results are in accordance with the findings of Uguen and colleagues (1999) who reported an increased in lacticin 481 productions due to increased in osmolarity of the growth medium after the addition of NaCl. Also, for plantaricin S, the highest production was observed at a sodium chloride concentration of 2.5% (wt/vol) (Leal-Sánchez et al., 2002). However, contrasting reports indicating the negative effect of NaCl are also available. The production of sakacin K by *L. sakei* CTC 494 has been shown to be negatively affected by NaCl (Leroy and DeVuyst, 1999), as is the case for the anti-listerial carnobacteriocin B2 produced by *Carnobacterium piscicola* A9b (Himelbloom et al., 2001). Production of the enterocins A and B by *Enterococcus faecium* CTC 492 is also inhibited in the presence of NaCl (Aymerich et al., 2000). Overall results indicate that lactocin RN78 and or its producer cells are more effective in the control and inhibition of *L. monocytogenes* in the cheese samples, compared to the action of NaCl. Temperature of storage appeared more and involved in the shelf life extension of these cheese samples. The bacteriocinogenic strains added to the cheese samples were more effective during storage at room temperature while all the rest of samples were more active at refrigerated temperature. However, cheese texture was greatly altered during storage at room temperature and spoiled much earlier compared to samples stored at refrigerated temperature. Besides temperature, pH also was altered in the samples. The increase in the pH recorded in these samples might be indicative of onset of proteolytic activity leading to the purification of the product. Our observations are in concordance with the results of Benkerroum (2003) who reported comparable results for bacteriocin producing *L. lactis subsp lactis* M studied in fermented sausages.

The effectiveness of lactocin producing *L. casei* RN 78 as an inhibitor of *L. monocytogenes* in experimental cheese samples could suggest its use in food system where post-manufacture contamination by this pathogen could be problematic. The ability of the bacteriocinogenic strain used in the study to grow in cheese sample and produces its bacteriocin and prevent the growth of *L. monocytogenes* appears interesting as it would not affect the manufacturing and the sensory characteristics of food. Furthermore, the ability of the producer cells to grow in cheese could be used to control the growth of advantageous bacteria which gives the manufacturer control of flavor development in cheese, and prevents economic losses which may occur if off flavors develop. Moreover, the stability of the freeze dried fractions of the bacteriocin in dry powder forms have the advantage that

it can be transported at low costs and have a longer shelf life.

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