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Screening for Bacteriocin-producing Lactic Acid Bacteria from Various Moroccan Food Products and Partial Characterization of Putative Bacteriocins

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Abstract: Screening for anti-*Listeria* bacteriocin-producing (Bac^+) lactic acid bacteria was carried out from different food products and the spectrum of action was determined against different Gram-positive and Gram-negative food borne bacteria of health and spoilage significance as well as closely related lactic acid bacteria. A total of 2242 strains randomly isolated from different foods were screened for the production of bacteriocin active against *L. monocytogenes*. The results showed that Bac^+ strains were more frequently isolated from dairy products (~95% of the positive isolates and 7.5% of overall dairy isolates) and that strains belonging to *Enterococcus*, *Lactococcus* and *Streptococcus* genera were the most represented with 76, 17.6 and 2.8% of the positive isolates, respectively. Conversely, Lactobacilli were not isolated from any of the tested dairy samples. While all strains were inhibitory to *L. monocytogenes* used as an indicator during the screening step, some of them were inhibitory to other pathogenic or spoilage Gram-negative or Gram-positive bacteria by the well diffusion assay.

Key words: Lactic acid bacteria, bacteriocin, *Listeria monocytogenes*, dairy, meat, vegetables

INTRODUCTION

Lactic Acid Bacteria (LAB) have a long history of safe use in fermented dairy, meat, fish or vegetable products. In fact, lactic fermentations are believed to be the oldest means of food preservation known to humankind (Ross *et al*., 2002). Such an application has been substantiated scientifically by establishing the ability of these bacteria to produce a variety of anti-microbial substances as a natural competitive means to overcome other microorganisms sharing the same ecological niche (Pattnaik *et al*., 2005).

Among the antimicrobial substances produced by LAB, bacteriocins have attracted increased interest of researchers and food producers during the last few decades. This interest has notably increased since early nineties due to the potential of bacteriocins or their producer strains as bio-preservatives and to the increased consumer preferences for foods without or with minimum chemical additives. Therefore, different bacteriocins produced by virtually all species of LAB have been characterized (Klaenhammer, 1993; Chen and Hoover, 2003). Bacteriocins or their producing LAB have been mainly used in food preservation and safety either separately or in combination with other conventional treatments (chemical additives or physical treatments) as part of the hurdle technology (Leistner, 1996). Other applications are now being considered including their use in functional foods (Prebiotics, synbiotics and probiotics or neutraceuticals) as well as in human therapy as antibiotics (Saarela *et al*., 2000).

L. monocytogenes is one of the pathogens of concern to food safety due its widespread presence in the environment, its high virulence and its resistance in stressful conditions (Thévenot *et al*., 2005). The latest report on risk assessment of *L. monocytogenes* in ready-to-eat foods of a joint FAO/WHO expert consultation (FAO/WHO, 2004) stated that though listeriosis is relatively rare and sporadic, it is a severe disease with a high fatality rate (20 to 30%).

L. monocytogenes has been shown to be sensitive to many bacteriocins produced by LAB. In particular, all bacteriocins of the lantibiotics and the sub class IIa are, by definition, inhibitory to *L. monocytogenes* (Klaenhammer, 1993; Cotter *et al*., 2005). Therefore, use of these antimicrobial substances or their producer strains as a biological means to enhance the control of specific food born pathogens is worthwhile considering and hence, search for new bacteriocin-producing LAB potentially useful to food preservation should continue.

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This study aimed to isolate as large as possible number of bacteriocin-producing LAB active against *L. monocytogenes* from different products and to partially characterize putative bacteriocins of selected strains that have a potential to be used in food biopreservation.

MATERIALS AND METHODS

Strains and culture conditions: The strains used in the present study, their origin and cultivation conditions are summarized in Table 1. A strain of *Listeria monocytogenes* ATCC 7644, a human clinical isolate, obtained from the American Type Collection Culture (ATCC), was used as the indicator bacterium during the screening lactic acid bacteria for bacteriocin production. All strains were stored at -80°C in their respective media [de Man, Rogosa and Sharp (MRS) borth (Oxoid, UK) for lactic acid bacteria and Tryptic soy broth (TSB; Biokar) otherwise] supplemented with glycerol (25%). Before use, strains were activated from the frozen stocks by two successive transfers in their respective broth media and incubated overnight at 37°C. Working cultures were maintained at 4°C on slants of MRS agar or Tryptic Soy agar (TSA; Biokar, France) and subcultured every two to three weeks.

Antimicrobial activity testing against *L. monocytogenes* ATCC 7644: Bacteria tested for bacteriocin production were isolated from different Moroccan food products including dairy, meat, vegetables, baking yeast and fish silage (Table 2). Sampling was conducted from 2000 to 2005 in a discontinuous way and some well characterized strains were published and assessed for their efficacy in food preservation (Benkerroum *et al*., 2000, 2002a and b, 2003). Samples (~250 g or mL each) were transported to laboratory of microbiology and biotechnology at Institut Agronomique et Vétérinaire Hassan II IAV Hassan II) of Rabat (Morocco) to carry out the screening for bacteriocin-producing LAB. Tenfold dilution series of each product were surface plated on MRS agar or on M17 agar (Terzaghi and Sandine, 1975) and then incubated for 24 h at 30°C. After incubation, colonies were randomly selected, grown in MRS broth, Gram stained and tested for catalase production. Gram positive and catalase negative strains were maintained as frozen stocks at -80°C in MRS broth with added glycerol (25%) until needed for antimicrobial activity testing. Bacteriocin producers were screened against *L. monocytogenes* ATCC 7644 by the well diffusion assay described by Tagg and McGiven (1971).

Table 1: Bacteria used in this study, their origin and cultivation conditions

Strains	Origin	Culture conditions (medium; incubation)
L. monocytogenes LMG15139	BCCM	TSB; 37°C, 24 h
L. monocytogenes LMG16783	BCCM	TSB; 37°C, 24 h
L. monocytogenes LMG13304	BCCM	TSB; 37°C, 24 h
L. monocytogenes ATCC 7644	ATCC	TSB; 37°C, 24 h
L. monocytogenes CWBI 2231	CWBI collection	TSB; 37°C, 24 h
L. monocytogenes M	CWBI collection	TSB; 37°C, 24 h
Micrococcus flavus 8166	IAV Hassan II collection	TSB; 37°C, 24 h
Staphylococcus aureus SAD30	IAV Hasan II collection	TSB; 37°C, 24 h
Staph. aureus CWBI	CWBI collection	TSB; 37°C, 24 h
Lactobacillus plantarum CWBI	CWBI collection	MRS; 37°C, 24 h
Lb. plantarum MD1	This study	MRS; 37°C, 24 h
Lb. curvatus CWBI-B28	This study	MRS; 37°C, 24 h
Lb. brevis CWBI	CWBI collection	MRS; 37°C, 24 h
Lb. curvatus CWBI	CWBI collection	MRS; 37°C, 24 h
L. lactis LMG 21215	BCCM	MRS; 37°C, 24 h
Streptococcus thermophilus B	This study	MRS; 37°C, 24 h
E. coli O78:K80 BJ2	IAV Hassan II collection	TSB; 37°C, 24 h
E. coli O157 VH21	Benkerroum *et al*. (2004)	TSB; 37°C, 24 h
Salmonella Enteri CWBI	CWBI collection	TSB; 37°C, 24 h
Sal. Typhi ST20	CWBI collection	TSB; 37°C, 24 h
Enterobacter cloacae B703	CWBI collection	TSB; 37°C, 24 h
Shigella sp. SI1	CWBI collection	TSB; 37°C, 24 h
Pseudomonas sp.	CWBI collection	TSB; 37°C, 24 h
Staphylococcus aureus-HG	CWBI	TSB; 37°C, 24 h
Lb. curvatus CWBI-B28	This study	MRS; 37°C, 24 h
Lb. curvatus CWBI-B28^{m}	This study	MRS; 37°C, 24 h
Lactobacillus plantarum-HG	CWBI	MRS; 37°C, 24 h
Lb. curvatus-HG	CWBI	MRS; 37°C, 24 h
Lactobacillus brevis-H28	CWBI	MRS; 37°C, 24 h
Escherichia coli-HG	CWBI	TSB; 37°C, 24 h
Pseudomonas sp. 55	CWBI	TSB; 37°C, 24 h

BCCM: Belgian Coordinated Collections of Microorganisms; ATCC: American Type Collection Culture; CWBI: Centre Wallon de Bio-Industrie; IAV Hassan II: Institut Agronomique et Vétérinaire Hassan II

Table 2: Screening for bacteriocin-producing lactic acid bacteria from different products

Products	Tested stains	Positive strains	Frequency of Bac$^+$ (%) A	B
Vegetables	330	0	0.0	0.00
Pickled green olive	130	0	0.0	0.00
Mixture of vegetables	200	0	0.0	0.00
Dairy products	1367	102	7.5	94.45
Cow milk	500	48	9.6	44.44
Camel milk	101	25	24.7	23.15
Sour camel milk	70	0	0.0	0.00
Raïb[1]	300	17	5.7	15.74
Lben[2]	180	12	6.7	11.11
Raw butter[3]	96	0	0.0	0.00
Jben	120	0	0.0	0.00
Meat products	340	2	0.6	1.85
Merguez[4]	160	1	0.6	0.93
Pork dry fermented sausages	180	0	0.0	0.00
Raw meat	40	1	2.5	
Miscellaneous	205	4	2.0	3.70
Baking yeast	75	4	5.3	3.70
Fish silage	130	0	0.0	
Total	2242	108	4.8	100.00

A:Percent of isolates from the same product; B: Percent of Bac$^+$ isolates; [1]A traditional Moroccan curdled raw milk by spontaneous fermentation. It is usually churned to separate Lben[2] from raw butter[3]; [4]Raw sausage made from lean and fat beef mixed with condiments

Confirmation of the bacteriocinogenic nature of the inhibitory substances: To confirm the bacteriocinogenic nature of the inhibitory substances produced by the putative bacteriocin-producing strains, additional tests were performed to exclude the effect of organic acids, hydrogen peroxide and to confirm the proteinaceous nature of the inhibitory substance and its bactericidal mode of action according to the techniques described below.

Elimination of the effect organic acids and hydrogen peroxide as inhibitory agents: Effects of organic acids were eliminated by adjusting the pH of the supernatants to 6.0 with 3 M NaOH. The supernatants were then filter-sterilized with a 0.22 mL Millipore filter membrane and subjected to anti-*Listeria* activity testing by the well diffusion assay.

To exclude the action of hydrogen peroxide, the NCFS was treated with catalase and assayed for inhibitory activity against *L. monocytogenes* as described by Barefoot and Klaenhammer (1983). Briefly, the cell-free supernatant was treated with 650 IU mL^{-1} of catalase in Tris HCl buffer (pH 8.0) for 2 h at 37°C. Controls were Tris HCl buffer with and without enzyme and non-treated cell free supernatant containing the same quantity of buffer as in the reaction mixture. Activity of treated supernatants was assayed by the well diffusion method.

Action of proteases: To confirm the proteinaceous nature of the inhibitory substances, the NCFS of a putative bacteriocin producer was treated separately with different proteases and then tested against *L. monocytogenes* ATCC 7644 by the well diffusion assay. Appropriate controls were run simultaneously. The proteases used were obtained from Sigma Chemical Company except trypsin, which was from Serva: trypsin (EC 3.4.21.4). All proteases were dissolved in phosphate buffer (0.1 M, pH 6) except pepsin which was dissolved in 0.02 N HCl solution (pH 2). Enzyme solutions were mixed with cell-free supernatants (1:1) to a final concentration of 1 mg mL^{-1}. Incubation was carried out at 37°C in a water bath for 2 h; then samples were boiled for 3 min to stop the reactions. Controls included sterile MRS broth, a mixture of culture supernatant with buffer (1:1) and buffer with only the enzyme. After boiling, each sample was assayed for bacteriocin activity by the well diffusion method.

Mode of action: The mode of action (i.e., bactericidal or bacteriostatic) was determined by monitoring the growth of *L. monocytogenes* ATCC 7644 in neutralized (pH 6) cell-free supernatants (NCFS) at 37°C. Alternatively, the method of Benkerroum *et al.* (1993) was used. Briefly, wells (6 mm in diameter) were punched in an overnight lawn of *L. monocytogenes* ATCC 7644 on M17 agar and filled with the NCFS to be tested then held at 4°C for 24-48 h. Development of clearing zones around the wells indicated that the antimicrobial substance diffuses through the agar medium and lyses the cells of the indicator strain. When no clearing was observed after 72 h, the final conclusion relied on the growth pattern of the sensitive strain in the NCFS.

Resistance to pH and heat: Effect of heat at various pH levels was examined by using two series of test tubes, each containing 4 mL of cell-free supernatant. In each series, the pH of the CFS was adjusted to 2, 6.0, 8.0 or 11 with 1 M HCl or 3 M NaOH. Unadjusted CFS (pH 4.2-4.5) was also used. After pH adjustment, one series was autoclaved (121°C for 15 min) and all samples (heated and unheated) were tested for anti-listerial activity by the well diffusion assay.

Identification of the bacteriocin-producing strains: The bacteriocin-producing strains were identified by physiological and biochemical tests (Harrigan and McCance, 1976; Manero and Blanch, 1999). The identity of selected strains of was confirmed by API 50 CH or by PCR based techniques.

Spectrum of action: The spectrum of activity against different bacteria of Gram positive and Gram negative (Table 1) was determined by the well diffusion assay

(Tagg and Mac Given, 1971). The producer strains were also tested against each other and against themselves.

RESULTS

Isolation of bacteriocin-producing lactic acid bacteria: A total of 2242 strains isolated from different products on a period span of 6 years starting from the year 2000 were examined for antilisterial activity at the laboratory of Food microbiology and biotechnology of the IAV Hassan II, Rabat (Morocco). The production of bacteriocin-like inhibitory substances was confirmed in the neutralized Cell Free Supernatants (CFS) of 108 (5.1%) isolates by the well diffusion assay after treatment with catalase and various proteases. The majority of the producers were isolated from dairy products (94.4%) with cow and camel's milk being the most common sources (Table 2).

Identification of bacteriocin-producing strains: The identification of the 108 bacteriocin-producing isolates showed a striking predominance of the genus *Enterococcus* (76%) represented by *E. facalis* (92.7 of the isolated enterococci) and *E. facium* (7.3%). Enterococci were particularly common in camel's milk where they were the only anti-*Listeria* bacteriocin-producing isolates. Among these isolates, *E. facalis* was, by far, the most predominant species (24 among 25 isolates). *Lactococcus* was the second most frequently isolated genus in dairy products with 17% of the bacteriocin-producing isolates and was represented by *Lc. lactis* subsp. *lactis*. Two *Leuconostoc* strains (1.9%) were isolated from dairy products and identified as *Leu. dextranicum* (Table 3). Two lactobacilli strains (CWBI-B28 and MD1) identified by a PCR-based technique as *Lb. curvatus* and *Lb. plantarum* were isolated from raw meat and merguez sausages, respectively (data not shown).

Sensitivity of the bacteriocins to proteases, heat and pH: A preliminary characterization of the bacteriocins produced by selected strains was performed on the basis of their sensitivity to different proteases, different pH and heat treatment at different pH values. The results summarized in Table 4 show that each inhibitory substance was sensitive to at least two proteases, which confirms their bacteriocinogenic nature according to widely accepted definition of Klaenhammer (1993).

Table 3: Identification of 108 anti-*listeria* bacteriocin-producing lactic acid bacteria from different products

	Species (%)[1]						
Products	*Leu. mesenteroides* subsp. *dextranicum*	*Lc. lactis*	*E. faecium*	*E. faecalis*	*Str. salivarius* subsp. *thermophilus*	*Lb. curvatus*	*Lb. plantarum*
Dairy products	2 (1.9)	19 (17.6)		76 (70.4)	1 (0.9)	0 (0%)	
Cow milk	-	12 (11.1)	1 (0.9)	35 (32.4)	-	-	-
Camel milk	-	-	-	24 (22.2)	1 (0.9)	-	-
Raïb	-	-	-	17 (15.7)	-	-	-
Leben	2 (1.9)	7 (6.5)	3 (2.8)	-	-	-	-
Meat products	0 (0%)	0 (0%)		0 (0%)		1 (0.9)	1 (0.9)
Merguez	-	-	-	-	-	-	1 (0.9)
Raw meat	-	-	-	-	-	1 (0.9)	-
Miscellaneous	0 (0%)	0 (0%)			2 (1.9)		
Baking yeast	-	-	2 (1.9)	-	2 (1.9)	-	-
Total	2 (1.9)	19 (17.6)	6 (5.6)	76 (70.4)	3 (2.8)	1 (0.9)	1 (0.9)

[1]Percent of the total bacteriocin-producing isolates

Table 4: Effect of protease treatment on the activity of antimicrobial substances produced by various strains of lactic acid bacteria isolated from different food products

		Protesase				
Strains	Source	Pronase E	α chymotrypsin	Trypsin	Pepsin	Papain
Lactococcus						
Lc. Lactis LMG 21215	Leben	S	S	S	R	S
Lc. lactis LMG 21206	Leben	S	S	R	R	ND
Enterococcus						
E. facium LMG21210	Leben	S	ND	S	R	ND
E. facium BSC5	Camel milk	S	S	S	S	ND
E. faecalis BSC15	Camel's milk	S	S	S	S	ND
E. faecalis HGR 47	Raïb	R	S	S	S	ND
Streptococcus salivarius* subsp. *thermophilus						
Str. thermophilus B	Baker's yeast	S	S	S	S	ND
Str. thermophilus HGV 54	Cow milk	S	R	R	S	ND
Lactobacillus						
Lb. curvatus CWBI-B28	Raw meat	S	S	ND	ND	S
Lb. plantarum MD1	Merguez	S	S	S	S	ND

S: Sensitive; R: Resistant; ND: Not Done

Differences between the antimicrobial substances sensitivity pattern to proteases has been observed suggesting that they are different bacteriocins.

As for the sensitivity to combined heat and pH, Table 5 shows that all bacteriocins tested remained active at low pH values with or without heat treatment except for the bacteriocin produced by *E. faecalis* BSC15 isolated from camel's milk which showed an opposite pattern being sensitive at low pH and resistant to autoclaving at neutral or alkaline pH. In this respect, this bacteriocin showed a similar pattern as diplococcin produced by members of *Lc. lactis* subsp. *cremoris* (Davey and Richardson, 1981).

Spectrum of action: The inhibitory activity of selected bacteriocin-producing strains was tested by the well diffusion assay against different Gram positive and Gram negative strains. The results (Table 6) showed that the

Table 5: Effect of pH and heat on the activity of putative bacteriocins produced by various strains of lactic acid bacteria

	Heated samples (121°C for 15 min)				Unheated samples			
Strains	pH 2	CFS[1]	pH 6	pH 11	pH 2	pH 6	pH 8	pH 11
Lactococcus								
Lc. lactis LMG 21215[2]	+	+	+	-	+	+	+	-
Lc. lactis LMG 21206[2]	+	+	+	-	+	+	+	-
Enterococcus								
E. facium LMG21210	+	+	+	-	+	+	+	-
E. facium BSC5	+	+	-	-	+	+	+	+
E. faecalis BSC15	-	+	+	+	-	+	+	+
E. faecalis HGR 30	+	+	+	-	+	+	+	+
Streptococcus salivarius* subsp. *thermophilus								
Str. thermophilus B	+	+	+	-	+	+	+/-	-
Lactobacillus								
Lb. curvatus CWBI-B28	+	+	+	-	+	+	+	+
Lb. plantarum MD1	+	+	+	-	+	+	+	+

+: Resistance to the treatment; -: Sensitivity to the treatment; +/-: Partially resistant (significant reduction of the diameter of the inhibition zone); [1]CFS = Cell free supernatant without pH adjustment; [2]Strains deposited in the Belgian Coordinated Collections of Microorganisms (BCCM)

Table 6: Spectrum of action of bacteriocin-producing strains of lactic acid bacteria and that of three antimicrobial peptides purified from the CFS of *Lactobacillus curvatus* CWBI-B28[1]

	Neutralized cell free supernatant			
Indicator strains	*L. lactis* LMG 21215	*Streptococcus thermophilus* B	*Lb. curvatus* CWBI-B28	*Lb. plantarum* MD1
Gram positive				
L. monocytogenes LMG15139	3+	3+	4+	3+
L. monocytogenes LMG16783	3+	3+	3+	3+
L. monocytogenes LMG13304	3+	3+	4+	3+
L. monocytogenes ATCC 7644	2+	3+	3+	3+
L. monocytogenes CWBI 2231	ND	ND	4+	ND
Micrococcus flavus NCIB8166	-	-	ND	ND
Staphylococcus aureus SAD30	-	2+	-	-
Staph. aureus CWBI	-	ND	-	ND
Lactobacillus plantarum CWBI	-	-	-	-
Lb. plantarum MD1	-	ND	-	-
Lb. curvatus CWBI-B28	-	ND	-	-
Lb. brevis CWBI	-	ND	-	ND
Lb. curvatus CWBI	ND	ND	3+	ND
L. lactis LMG 21215	-	-	3+	3+
Staphylococcus aureus-HG	ND	ND	ND	ND
Lb. curvatus CWBI-B28	ND	ND	ND	ND
Lb. curvatus CWBI-B28[m]	ND	ND	ND	ND
Lactobacillus plantarum-HG	ND	ND	ND	ND
Lb. curvatus-HG	ND	ND	ND	ND
Lactobacillus brevis-H28	ND	ND	ND	ND
Gram negative bacteria				
E. coli O78:K80 BJ2	-	ND	3+	3+
E. coli O157 VH21	-	ND	3+	3+
Salmonella Enteri CWBI	-	1+	-	3+
Sal. typhi ST20	-	2+	ND	3+
Enterobacter cloacae B703	-	2+	ND	ND
Shigella sp. SI1	-	2+	ND	ND
Pseudomonas sp.	ND	ND	2 +	ND
Escherichia coli-HG	ND	ND	ND	ND
Pseudomonas sp. 55	ND	ND	ND	ND

4+: Diameter of the inhibition zone >25 mm; 3+ 16 <Φ< 22 mm; 2+ 12 <Φ< 20 mm; - : No inibition; ND = Not done; [1]Each of three purified antimicrobial peptides inhibited sensitive strains to the same extent when tested separately

spectrum of action of the lactococcal strain was restricted to *Listeria* strains which is one of the features that characterizes the class IIa and lantibiotic type bacteriocins according to the classification of Klaenhammer (1993). However, *Str. thermophilus* and the two lactobacilli strains inhibited, to different extents, Gram negative strains of spoilage or health significance. Though many authors have previously suggested the inhibition of Gram negative bacteria by some bacteriocins produced by LAB, such an issue remains controversial. Therefore, we attempted to confirm these data on the purified or the partially purified bacteriocins and the inhibitory activity against Gram negative bacteria was lost upon purification (data not shown).

DISCUSSION

The present study showed that dairy products, especially raw milk is one of the primary sources to isolate LAB producing bacteriocins active against *L. monocytogenes*. This result confirms the high incidence of bacteriocin-producing lactic acid bacteria in milk products reported in other studies (Rodriguez *et al*., 2000). However, Ennahar *et al*. (1999) reported that the most bacteriocin-producing strains, particularly those producing class IIa bacteriocins, have been isolated from meat products. In addition, isolation of bacteriocin-producing strains from vegetable products is less frequently reported than their isolation from dairy and meat products. The ability of a strain to produce bacteriocin may be related to its capacity to withstand adverse conditions prevailing in its natural niche or to the complexity of its microbiota where only the most competitive strains survive. In effect, it has been extensively reported that environmental factors including stressful conditions are the key parameters that influence the magnitude of the production of antibacterial substances by bacteria as a means to overcome competitive strains living in the same environment (Pattnaik *et al*., 2005). Among the bacteriocin-producing strains isolated in this study, enterococci were the most common followed by lactococci in accordance with the results reported previously by Rodriguez *et al*. (2000). The predominance of enterococci strains in dairy products was extensively reported particularly in those produced in Mediterranean countries (Bouton *et al*., 1998). Furthermore, it has often been reported that *Enterococcus feacalis* and *E. faecium* strains are able to produce antilisterial bacteriocins when grown in milk and dairy products (Giraffa and Carminati, 1997). Despite the high number of strains isolated from dairy products, no bacteriocin-producing lactobacilli were detected in these products reflecting the low frequency of this genus in Moroccan dairy products as has been previously reported (Benkerroum and Tamime, 2004a).

Despite the high number of bacteriocin-producing LAB isolated and characterized so far, further search for new strains belonging to all genera of LAB having different spectra of action and isolated from different environments is worthwhile. According to Klaenhammer (1988), 99% of all bacteria may make at least one bacteriocin. The trend of consumer preferences for the authentic foods or the minimally processed food and free from chemical additives gears towards the use of the multiple hurdles technology based on biological means, including the use of bacteriocins or their producer strains, to ensure food safety and keeping quality. In Europe, levels of lactic acid bacteria in raw milk have decreased due to the extent of refrigeration and to the application of stringent hygiene standards. In this regard, some bacteriocin-producing strains isolated in this study were tested for their antilisterial activity in situ in meat (Benkerroum *et al*., 2003, 2005; Ghalfi *et al*., 2006b), fish (Ghalfi *et al*., 2006a) or dairy (Benkerroum *et al*., 2000) products and have reduced *Listeria* counts to different extents depending on the products and the Bac^+ strains. On the other hand, the number of strains used in industrial fermentations is relatively low. For this reason, the characterization of lactic acid bacteria from food products manufactured by traditional techniques without commercial starter cultures in order to extend the number of available cultures has been considered in the search for industrially important characteristics (Cogan *et al*., 1997). Moreover, in the scope of technology transfer of traditional foods, especially the fermented type, to industrial scale there is a need to search for more appropriate strains to be used in the starter cultures for such a transfer to be successful. Hence, the strains to be used in the starter culture should be selected not only on the basis of their technological performances but also taking into account other properties including the ability to enhance the safety and stability of the end product. Availability of the widest possible range of bacteriocin-producing strains of LAB will help promoting the technology transfer of some traditional fermented products in developing countries so they can keep their authenticity in compliance with the food safety requirements.

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