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Isolation and identification of *Leuconostoc mesenteroides* producing bacteriocin isolated from Algerian raw camel milk

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Eighty three strains of *Leuconostoc mesenteroides* were isolated from 12 samples of raw camel milk collected from different Algerian zones. Based on morphological, biochemical and physiological characters tests, 36.52% strains were identified as *L. mesenteroides* spp. *mesenteroides,* 30.71% as *L. mesenteroides* spp. *dextranicum* and 3.32% as *L. mesenteroides* spp. *cremoris*. The interactions study revealed that seven strains were able to inhibit the growth of *Listeria monocytogenes, Listeria innocua, Listeria ivanovii, Staphylococcus aureus, Escherichia coli* and *Lactobacillus plantarum*. Setting aside the pH effect, H_2O_2 effect and using proteolytic enzymes, the inhibitory agent was determined as proteinous nature substance. Treatment of the cell-free supernatant of the bacteriocin-producing strains by Tween 80 increased the inhibition activity, contrary to the urea which decreased it.

Key words: Raw camel milk, *Leuconostoc mesenteroides*, *Listeria* spp., bacteriocin, biopreservation.

INTRODUCTION

In conditions of extreme drought and lack of pasture, unlike other animals, female camels (*Camelus dromedarius*), are able to produce high quality of milk (Yagil and Etzion, 1980; Schwartz, 1992) and, therefore, contribute significantly to the food security of the nomads (Ahmad et al., 2010). Camel milk, generally opaque and white, has an acceptable taste, it is considered to have anti-cancer (Magjeed, 2005), hypo-allergic (Shabo et al., 2005) and anti-diabetic properties (Agrawal et al., 2003). Raw camel milk could be an additional source of typical dairy lactic acid bacteria (LAB) species (Khedid et al., 2009). The selection of strains from dairy ecosystems other than cow's milk and cheeses provides an opportunity to obtain strains which possess unique phenotypes (Drici et al., 2010).

The LAB, frequently termed “the lactics”, constitute a diverse group of microorganisms associated with plants, meat, raw milk and dairy products (Carr et al., 2002; Azadnia et al., 2011).

LAB are best known for their use as starter cultures in the manufacture of dairy products and have beneficial effects in the food industry, they can be a nuisance as contaminants by producing off-flavors and increase shelf life (Abee, 1995; Cai et al., 1998; Hugenholtz and Kleerebezem, 1999).

Currently, the lactic acid bacteria include thirteen different bacterial genera: *Lactobacillus, Leuconostoc, Lactococcus, Streptococcus, Enterococcus, Pediococcus, Bifidobacterium, Carnobacterium, Oenococcus, Weissella, Aerococcus, Tetragenococcus* and *Vagococcus* (Dortu and Thonart, 2009).

Among the LAB, the genus *Leuconostoc* is present in many environments such as milk and dairy products, green vegetation, wine and meat. It can easily spread

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Table 1. Characteristics of the indicator bacteria.

Indicator strain	Origin	Code	Growth
Listeria monocytogenes	CECT (Spain)	ATCC 4032	BHIB /37°C
Listeria ivanovii	CECT (Spain)	ATCC 19119	BHIB /37°C
Listeria innocua	CECT (Spain)	ATCC 33090	BHIB /37°C
Staphylococcus aureus	Applied microbiology laboratory (Algeria)	ATCC 43300	Nutrient broth /37°C
Escherichia coli	Applied microbiology laboratory (Algeria)	ATCC 25922	Nutrient broth /37°C
Lactobacillus plantarum	Applied microbiology laboratory (Algeria)	58	Nutrient broth /37°C

in fermented products (Hemme and Foucaud-Scheunemann, 2004). They are involved in a large number of spontaneous fermentations of food products, which led to the recognition of their status as Generally Recognized As Safe (GRAS) (Klaenhammer et al., 2005) and approved by the qualified presumption of safety (QPS) for food production and human consumption (Leuschner et al., 2010). *Leuconostoc* strains are mesophilic, Gram-positive, catalase negative, non-motile and aero-tolerant bacteria unable to metabolize arginine. They are obligatory heterofermentative cocci, often ellipsoidal. They usually occur in pairs and chains (Hemme and Foucaud-Scheunemann, 2004; Holland and Liu, 2011). The Leuconostoc genus comprises *Leuconostoc mesenteroides* (with the three subspecies, *mesenteroides, dextranicum and cremoris*) and 13 other species, *Leuconostoc citreum, Leuconostoc carnosum, Leuconostoc durionis, Leuconostoc fallax, Leuconostoc ficulneum, Leuconostoc pseudoficulneum, Leuconostoc fructosum, Leuconostoc gasicomitatum, Leuconostoc gelidum, Leuconostoc inhae, Leuconostoc kimchii, Leuconostoc lactis, Leuconostoc pseudomesenteroides* (Ogier et al., 2008).

Interestingly, the genus includes good antagonist activity, although it has also been linked to the deteriorating food activities. The antimicrobial action of *Leuconostoc* against micro-organisms and alteration has been attributed to different mechanisms including bacteriocins (Ennahar et al., 2000; Cocolin et al., 2007).

These substances exhibit bactericidal activity against species closely related to the producer strain (De Vuyst et al., 1994). These compounds (bacteriocins) are peptides or proteins that have antimicrobial activity. They are divided into four classes based on common characteristics, mainly primary structure, molecular weight, mode of action, heat stability and their genetic properties. Among these classes, class II, consisting of small peptides that do not contain modified residues, has been divided further into subgroups. Class IIa bacteriocins show their strong inhibitory effect on *Listeria* sp. (Yanhua et al., 2012) especially against *Listeria monocytogenes* (Trias et al., 2008; Alcina et al., 2002) as well as other food spoilage and pathogenic bacteria, so they are considered as potential natural food preservatives (Ennahar et al., 1999; Vermeiren et al., 2004; Gálvez et al., 2007).

The aim of this work was to isolate and characterize of anti-listerial *L. mesenteroides* from Algerian raw camel milk.

MATERIALS AND METHODS

Bacterial strains and growth media

The chosen bacteria as indicators were *Listeria innocua* ATCC 33090*, Listeria ivanovii* ATCC 19119 and *L. monocytogenes* CECT 4032 (CECT, Spain), *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 43300 and *Lactobacillus plantarum 58* (Applied microbiology laboratory, Oran, Algeria). The conservation of the indicator microorganisms and the *L. mesenteroides* strains were kept at -20°C in nutrient broth and sterile skim milk containing 0.25% yeast extract and 0.5% glucose, respectively, both were supplemented with 20% of glycerol.

Working cultures were conserved as slants at 4°C in MRS (De Man Rogosa and Sharpe) agar containing 1% lactose for the *L. mesenteroides* strains and in nutrient agar for the indicator bacteria. Before experimental use, aliquots were enriched twice into the appropriate medium and incubated to the conditions shown in Table 1.

Samples

Twelve raw camel milk's samples were obtained from different Algerian arid zones (Table 2) and were analyzed. The samples were collected in sterile glass bottles; they were transported to the laboratory in a cool box and stored at 4 ± 1°C until analysis within 12 to 30 h.

Isolation and identification of *L. mesenteroides*

In order to isolate *L. mesenteroides* from raw camel milk, 1 ml of each sample was aseptically transferred into 9 ml of sterile saline solution (0.9% sodium chloride solution, pH 7) (w/v) to make an initial dilution (10^{-1}). Serial dilutions were made for each sample and 1 mL of the appropriate dilution (10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}) was spread respectively in duplicate into plates containing a selective medium, MRS (pH 6.8) supplemented with 30 µg/ml of vancomycine (Mathot et al., 1994) and MSE medium (Mayeux et al., 1962) which was used to detect producing Dextrane *Leuconostoc* strains.

Twenty isolated strains were randomly selected from each sample; and were purified by subculturing on MRS and MSE agar plates followed by microscopic examination. The purified isolates were initially tested for Gram stain, cell morphology, H_2O_2 production and spore formation. Strains with positive Gram, diplococcic shape, catalase negative and non-spore formation were selected for further identification. The strains were tested for physiological characters as CO_2 production from glucose, growth at different temperatures (4, 15, 30, 37 and 45°C) for 5 days and at different pH (4.8 to 6.8), resistance to different NaCl concentrations (3 to 6.5%), thermo-tolerance was carried out at 63.5°C for 30 min and at 55°C

for 10 min; and biochemical tests such as: dextran production from sucrose, arginine hydrolysis (ADH), citrate degradation, production of acetoine were carried out. In order to differentiate the subspecies of *Leuconostoc* strains, carbohydrate fermentation profile was then performed.

The CO_2 production was evaluated in MRS broth containing inverted Durham tubes, the positive reaction was visually confirmed by the filling of the Durham tube with CO_2 which led to the increase of the latter. The growth of strains in different temperatures, pH, NaCl concentrations and thermo-tolerance was observed by turbidity of the broth medium. As for the dextran production, strains were seeded on the MSE medium containing 10% of sucrose to synthesize polysaccharides (dextran). Arginine hydrolysis was searched on M16 BCP medium which contains lactose, arginine and Bromocresol purple as an indicator of pH. Positive reaction of the degradation of citrate was performed on the KMK medium (Kempler and Mackay) as interpreted by the appearance of blue green colonies. Acetoin production from glucose was determined using the Voges-Proskauer test on Clark and Lubs broth. The fermentation profile testing was carried out on MRS-BCP broth (10 g polypepton, 5 g yeast extract, 1.08 g Tween 80, 2 g K_2HPO_4, 5 g sodium acetate, 2 g ammonium citrate, 0.2 g $MgSO_4$, 0.05 g $MnSO_4$ and Bromocresol purple) without carbon source; the culture medium was supplemented with 3% (w/v) of the following carbon sources previously sterilized by filtration: arabinose, maltose; rhamnose, manitol, sorbitole, galactose, lactose, fructose, glucose, sucrose, xylose, tréhalose, raffinose.

Antimicrobial activity detection

Antimicrobial activity of *L. mesenteroides* strains toward indicator bacteria was performed by two methods: the spot agar test (direct method) as described by Tagg and McGiven (1971) and the agar well diffusion assay (indirect method) as described by Schillinger and Luck (1989).

For the direct method, colonies of *L. mesenteroides* were spotted into MRS agar (0.75%) and incubated at 30°C; 100 µl of an 18 h culture of the indicator bacteria was transferred into 10 ml of Mueller-Hinton (MH) agar then poured over the spots. After incubation, the indicator bacteria were examined for their zones of inhibition.

In the indirect method, cell-free culture supernatants of the *L. mesenteroides* grown in MRS broth for 24 h (inoculum 1%, v/v, 30°C) were obtained as obtained by Lacroix and Millete (2011) by removing cells by centrifugation (8000 rpm for 30 min at 4°C) then adjusted to pH 6.5 by 1 M NaOH and sterilized by filtration through 0.45 µm Millipore filter. Supernatant (80 µl) was transferred delicately into 8 mm holes drilled into MH agar medium inoculated (1%, v/v) with indicator strains. The plates were incubated at 37°C for 24 h.

Antimicrobial activity in both methods was recorded as positive if there were translucent halo around the well.

Effect of enzymes, pH and heat treatment

The sensitivity of the active substance to proteolytic enzymes was tested on supernatant fluids (as described above, indirect method). 100 µl aliquots of supernatant were treated respectively with 1 $mg.ml^{-1}$ as final concentration of pepsin and α-chymotrypsin for 2 h at 36 ± 1°C and then 10 min at 65°C was performed to stop the reactions before the antimicrobial activity assay was done.

Supernatant without treatment was used as control. The effect of pH was estimated by adjusting pH of the supernatant fluids to the following values 2, 4, 6, 8 and 10 using HCl or NaOH. Supernatant fluids pH 6.5 was used as control. Thermo-stability of the antimicrobial activities was determined by heating supernatant fluids at range of 30 to 121°C for 15, 30 and 60 min. Samples without heating were used as control.

Optimization of the antimicrobial activity using Tween 80 and urea

Cell-free culture supernatants of the *Leuconostoc* strains grown for 24 h at 30 °C were obtained by removing cells by centrifugation (8000 rpm for 30 min at 4°C) (Lacroix and Millette, 2011) neutralized by NaOH and sterilized by filtration through 0.45 µm pore filter. Supernatants were treated by Tween 80 and urea at final concentration of 1% by incubating supernatant fluids for 2 h at 36 ± 1°C. The activity was measured by the agar well diffusion assay.

RESULTS

Sample collection

A total of 12 samples of raw camel milk were collected from different Algerian arid zone (Bechar, El Bayad, El Aghwat, Tindouf...) in different season (March 2011 to May 2012). Characteristics of samples of raw camel milks are shown in Table 2. The milk was collected in sterile glass bottles; most of them were transported by air plane to the laboratory in a cool box and stored at 4 to 6°C before analysis. The samples were analyzed within 12 to 30 h of collection.

Isolation and identification of microorganisms

Eighty three strains of *L. mesenteroides* were isolated from 12 raw camel milk samples. All the 83 isolates were Gram positive, ovoid shape associated in pairs and/or short chains, negative catalase reaction, able to produce carbon dioxide from glucose and unable to hydrolyze Arginine. All the isolated strains were grown at 15, 30 and 37°C but not at 4 and 45°C. pH 6.5 was suitable for our isolates contrary to pH 4.8. 87% of the strains were able to grow at 3% NaCl concentration however 18% resisted the concentration of 6.5% of NaCl. The totality of our strains tolerated 55°C for 15 min and none of them was able to resist at 63.5°C for 30 min. 77% of the isolates were able to hydrolyze citrate, 48% produced acetoine and only 6 strains did not produce dextran from sucrose. Concerning the carbohydrate fermentation profile, all the isolates used glucose, galactose, lactose, maltose, fructose as carbon source however they did not use raffinose, rhamnose, sorbitol and starch, there was a variability result of using mannose, sucrose, xylose and D-Arabinose. Table 3 shows biochemical and physiological characteristics of 7 strains of bacteriocin-producing *L. mesenteroides (Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln* C29 and *Ln*C33).

Inhibitory activity and spectrum

The isolated *L. mesenteroides* were found to possess antibacterial activity. From 83 isolates, we retained 7

Table 2. Raw camel milk characterization.

Sample	Region, Wilaya	Camel's tint	Age (year)	Camel's breed	Sampling time	Daily production (L)
1	Abadla, Bechar	Brown	5	Reguibi	19h :30	6 – 9
2	Abadla, Bechar	Brown	9	Reguibi	19h :30	6 – 9
3	Sidi Makhlouf, laghouat	Brown	10	Ouled Sidi Cheikh	7h :40	5 – 10
4	Zouzfana, Bechar	yellowish	9	Reguibi	14h :00	6 – 9
5	Zouzfana, Bechar	Brown	5	Reguibi	6h :00	6 – 9
6	Wadi Chergui, El Bayed	yellowish	7	Berberi	6h :20	4 – 7
7	Beni mellal, Abadla, Bechar	yellowish	4	Reguibi	17h :00	5 – 10
8	nomad, laghouat	yellowish	8	Ouled Sidi Cheikh	/	5 – 8
9	nomad, laghouat	Brown	7	Ouled Sidi Cheikh	/	5 – 8
10	El mora, abadla	Brown	8	Reguibi	19h :30	6 – 10
11	nomad, Tindouf	Brown	5	Targui	18h :15	6 – 9
12	nomad, Tindouf	yellowish	6	Targui	18h :15	6 – 9

Table 3. Biochemical and physiological characteristics of the bacteriocin-producing *L. mesenteroides* strains.

	***Ln*C12**	***Ln*C21**	***Ln*C23**	***Ln*C26**	***Ln*C28**	***Ln*C29**	***Ln*C33**
Arginine hydrolysis	-	-	-	-	-	-	-
CO_2 production	+	+	+	+	+	+	+
Dextran production	+	+	+	+	+	+	+
Citrate utilization	-	+	+	+	+	+	+
Acetoin production	+	-	-	+	+	-	-
3% NaCl	+	+	+	+	+	+	+
6.5% NaCl	-	-	-	-	-	-	-
T° 4°C	-	-	-	-	-	-	-
T° 15°C	+	+	+	+	+	+	+
T° 37°C	+	+	+	+	+	+	+
T° 45°C	-	-	-	-	-	-	-
55°C/15 min	+	+	+	+	+	+	+
63.5°C/30 min	-	-	-	-	-	-	-
pH 4	-	-	-	-	-	-	-
pH 6.5	+	+	+	+	+	+	+
Substrate fermentation							
Glucose	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
Mannose	±	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Xylose	+	+	±	+	+	±	+
Raffinose	-	-	-	-	-	-	-
D-Arabinose	-	-	-	+	+	-	-
Sorbitol	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-

Symbols: +, positive reaction, -, negative reaction, ±, non-complete degradation.

Table 4. Spectrum activity of the bacteriocin-producing *L. mesenteroides* strains isolated from raw camel milk.

Strain	*Listeria ivanovii*		*Listeria innocua*		*Staphylococcus aureus*		*Escherichia coli*		*Lactobacillus plantarum*	
	MRS	MRSB	MRS	MRSB	MRS	MRSB	MRS	MRSB	MRS	MRSB
*Ln*C12	8.7±0.07	6.3±1.56	4.5±0.11	3.8±0.07	8	5	5.8±0.05	3	8.8±0.5	6.5±0.5
*Ln*C21	8.6±0.05	5.6±0.07	3	2.5±0.13	7	4.3±0.03	3	1.3±0.03	7	5
*Ln*C23	7.1±0.14	5	2.3±0.55	1.6±0.21	8.6±0.23	4.6±1.02	3.6±0.02	3	6.3±	3
*Ln*C26	7.5±0.7	4.5±0.80	3.5±0.25	1	7.3±1.02	3	3.3±1.28	2.8±1.01	7.5±0.02	2.8±1.34
*Ln*C28	6	4	2	1±0.93	5	2.1±0.23	4.6±0.14	3.5±0.06	6	3.8±2.01
*Ln*C29	6.8±0.03	3.3±0.48	2.6±0.04	1±0.82	7.6±0.22	2.4±0.35	3.5±0.11	1	7.3±0.1	3
*Ln*C33	5.01±0.5	2.6±0.24	3.5±0.51	1±1.75	5.7±0.1	2	3.3±0.09	2.5±0.02	7	2.6±1.25

MRSB: MRS medium buffered.

strains (*Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33) that showed an inhibitory activity against *Listeria* spp. including *L. monocytogenes* (data not shown). Activity of the neutralized cell-free supernatant was evaluated against food born pathogen and food spoilage bacteria. The strains presented in Table 4 showed remarkable inhibition spectra against the latter. The obtained results are shown in Table 4 and Figure 1.

The diameters of inhibition are between 8.8 ± 0.5 mm and 1 mm. The highest diameters noticed were 8.8 ± 0.5 mm against *L. plantarum* and 8.7±0.07 mm against *L. ivanovii* of the strain *Ln*C12, as for the lowest diameters were obtained with the two strains *Ln*C28 and *Ln*C33 against *L. innocua* and *S. aureus*, respectively.

The average diameters of *L. mesenteroides* strains *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33 against *L. ivanovii, L. innocua, S.aureus, E. coli* and *L. plantarum* revealed various diameters but lower than the strains *Ln*C12, *Ln*C21 and *Ln*C23.

The most inhibited indicators strains are the most part of Gram positive bacteria (*L. ivanovii, L. innocua, S. aureus* and *L. plantarum*); a single gram negative indicator bacteria (*E. coli*) was inhibited by our strains.

Characterization of the inhibitory substance

Antagonistic activity of the antimicrobial compounds was abolished to the strains *Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33 after treating the cell-free supernatant with 1% of pepsin and α-chymotrypsin.

The effect of temperature, pH and organic solvents on the antimicrobial activity is shown in Table 5. The bacteriocin activity of our 7 bacteriocin-producing *L. mesenteroides* strains (*Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33) remained unchanged after incubation at 50°C for 60 min but some loss was observed after incubation at 60°C for 30 min. At 100°C for 30 min, the residual antimicrobial activity decreased for *Ln*C12, *Ln*C21 and *Ln*C23. However, no activity was observed for the other strains. Total inactivation was observed of the activity by heating the cell-free supernatant to 100°C for 60 min. pH effect on the antimicrobial activity was stable after incubation for 2 h in pH values ranging from 4 to 8. However, no activity was observed in pH 2 and 10.

The effect of organic solvents is shown on Table 5 and Figure 2. Treatment of the cell-free supernatant with urea reduced the activity of our *L. mesenteroides* strains; however treatment with Tween 80 increased the inhibition spectra.

DISCUSSION

In this study, 83 strains of *L. mesenteroides* were isolated from twelve different raw camel milk collected from different Algerian arid zones. According to the previously published phenotypic tests (Gonzalez et al., 1990) based on diagnostic characteristics of the species of the genus *Leuconostoc* outlined by Garvie (1986), strains were allotted to the genus *Leuconostoc.* On the other hand, physiological and biochemical tests were performed; according to Badis et al. (2005) and Ogier et al. (2008), 42 strains were identified as *L.mesenteroides* spp. *mesenteroides*, 37 strains as *L. mesenteroides* spp. *dextranicum* and 4 strains as *L. mesenteroides* spp. *cremoris*. The presence of *Leuconostoc* genus in camel milk was confirmed by several authors where Benkerroum et al. (2003) got 1%, Nurgul et al. (2009) got 10% and Khedid el al. (2009) got 11.7% from their total isolated strains.

After identification of the isolates, detection for antagonistic activity was done by the spot agar test, our results showed good inhibition spectra on non buffered MRS agar medium but there was a reduction in spectra using buffered medium, which was due to lactic acid production, then the effect of acidity was eliminated by adjusting pH of the cell free supernatant to pH 6.5. The antagonistic effect of the antibacterial action cannot exclude the effect of acids (Deegan et al., 2006), hydrogen peroxide (Piard et al., 1991), diacetyl (Condon et al., 1987) or bacteriocin-like substances (Alexandre et al., 2006) by the strains of *Leuconostoc.*

Our obtained results indicate that *L. mesenteroides* strains are capable of producing inhibitive substances

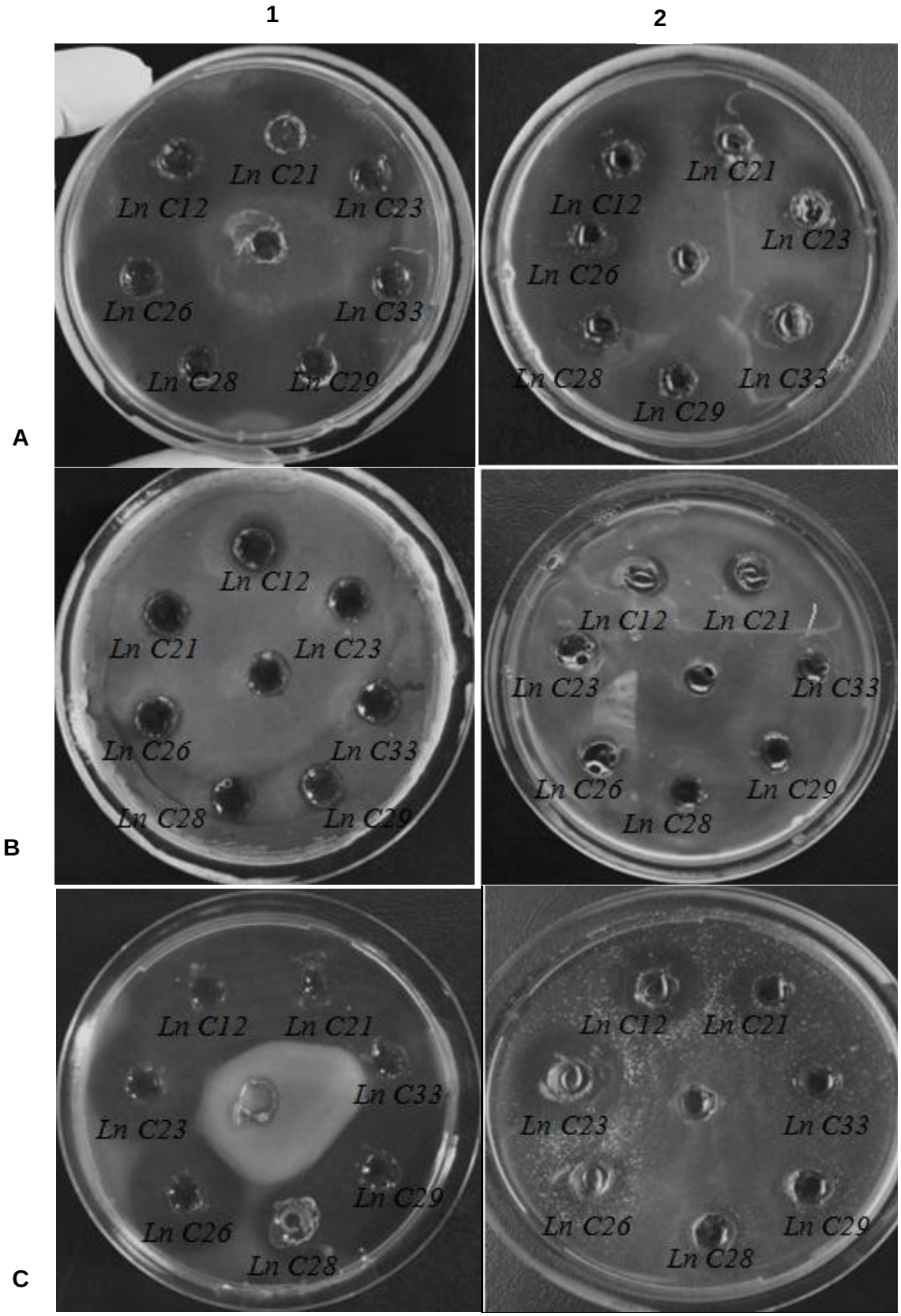

Figure 1. Testing cell-free supernatant using agar well diffusion assay of *Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33 for indicator strains. A) *Listeria ivanovii*, B) *Listeria innocua*, C) *Staphylococcus aureus*, D) *Escherichia coli*, E) *Lactobacillus plantarum*; 1: MRS medium, 2: buffered MRS medium.

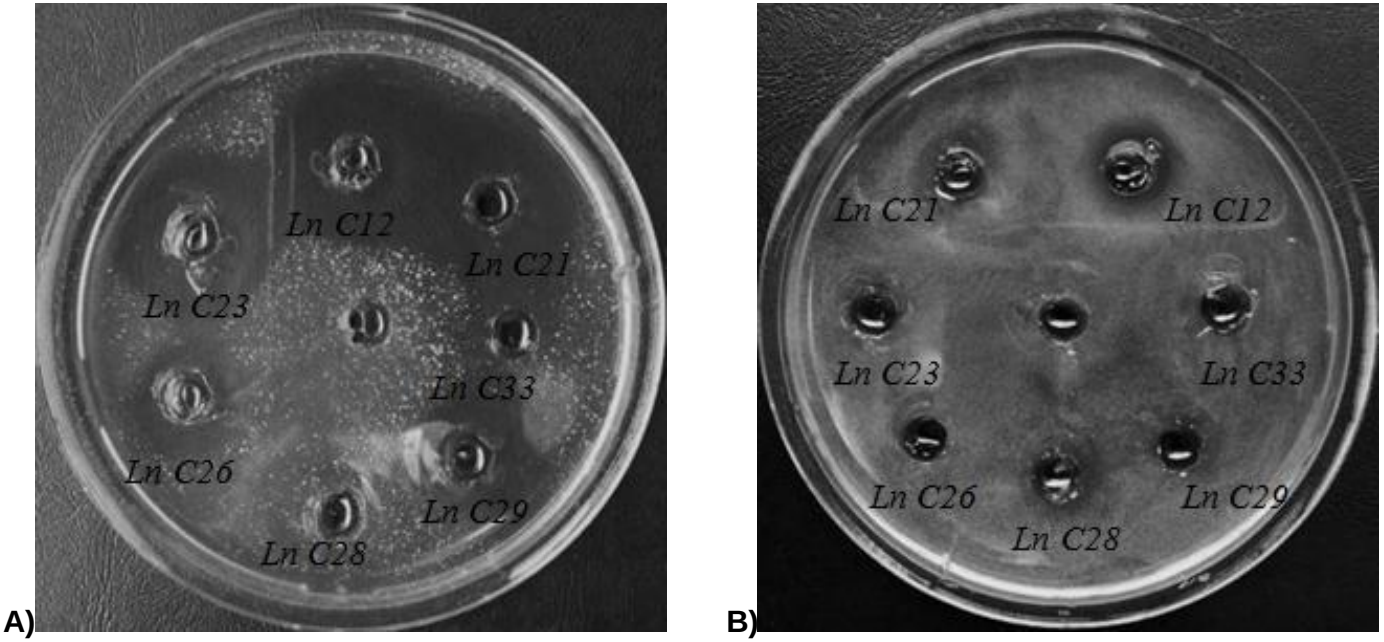

Figure 2. Effet of organic solvents on the cell free supernatant of the strains *Ln*C12, *Ln*C21, *Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 *and Ln*C33. A) Treatment by Tween 80, B) treatment by urea.

Table 5. Influennce of temperature, pH, proteases and organic solvant.

Strains	Temperature					pH					Proteolytic enzymes		Organic solvant	
	50°C/60 min	60°C/30 min	100°C/15 min	100°C/ 30 min	100°C/ 60 min	2	4	6	8	10	α-Chymotrypsin	pepsin	urea	Tween80
Ln C12	6.3±0.13	4	3.5±0.5	1	0	0	6	6.5±0.03	4.3±0.90	0	0	0	9.7±0.27	3±0.23
Ln C21	5.1±0.35	3	2	0.5±0.31	0	0	4.9±0.26	5	3.7±0.75	0	0	0	9.6±0.9	2
Ln C23	5	3.6±0.65	3.8±0.22	1.4±0.15	0	0	5.3±0.12	5.81±0.07	4±0.5	0	0	0	8	2.5±0.8
Ln C26	4.6±0.29	3.3±0.78	2	0	0	0	4	4.09±0.18	3	0	0	0	7.7±0.48	2
Ln C28	4.5±1.13	2.5±1.1	2.5±0.29	0	0	0	4.2±0.07	3.43±1.01	1.9±1.22	0	0	0	5.2±0.43	1.9±1.11
Ln C29	3.3±0.21	1.9±0.17	1	0	0	0	3.7±1.52	3	0.9±0.34	0	0	0	3	0
Ln C33	2.8±2.5	1	1.7±0.36	0	0	0	2±0.96	2.5	0.7±0.89	0	0	0	1	0

that have bactericidal effect on pathogenic bacteria. These inhibitive substances produced by our seven *L. mesenteroides* strains (*LnC12, LnC21, Ln*C23, *Ln*C26, *Ln*C28, *Ln*C29 and *Ln*C33) act differently on the pathogenic indicators strains.

The cultures were checked for bacteriocin pro-

production using the agar well diffusion assay; our obtained results showed that 8% of 83 isolated strains gave inhibition zones against *L. ivanovii, L. innocua, S. aureus, E. coli* and *L. plantarum*. Lewus et al. (1991) found that only a few of *L. mesenteroides* tested for antagonistic activity were positive using the spot agar method which can give positive results in the well diffusion assay. The results of the well diffusion method showed that *L. ivanovii, S. aureus* and *L. plantarum* were the most sensitive to our retained *L. mesenteroides* strains. Gram positive indicator bacteria are much more sensitive to the inhibitory substance produced by our strains than Gram negative indicator bacteria.

The sensitivity of the found inhibitory antibac-terial substance to proteinase α-chymotrypsin and pepsin is a proof of its proteinaceous nature, which allows considering it as a bacteriocin.

In the present study, *L. mesenteroides* strains bacteriocin retain activity at range of pH (4 to 8). The stability at low pH was very important for their potential application in foods, such as fermented products, in which acidic conditions prevail. They were also quite heat stable, since 57% loss of activity of *L. mesenteroides* bacteriocin was noticed after incubation at high temperatures (100°C for 30 min). Other bacteriocins isolated from *L. mesenteroides* have similar features. Enterocin 1146 (Parente and Hill, 1992) was also described as heat stable, although, it was partially or totally inactivated at temperatures above 60°C.

Treatment by the addition of Tween 80 had a positive effect on the antimicrobial activity by increasing the bacteriocin production. Being a surfactant, Tween 80 may prevent the adsorption of the bacteriocin to the producer cell and this might be due to a stabilization of a favourable configuration of the bacteriocin molecules (Huot et al., 1996). The maintenance of the activity after the addition of Tween 80 has been observed for lactacin F from *Lactobacillus* (Muriana and Klaenhammer, 1991). The good effect of Tween 80 on our strains might be due to a stabilization of a favorable configuration of the bacteriocin molecules (Huot et al., 1996).

In our study, in the cell-free supernatants from seven strains of *L. mesenteroides,* the antibacterial substance exhibited moderate action against food-borne pathogen *Listeria* spp. including *L. monocytogenes*. Therefore, could be employed as starter or protective cultures in food industry. Since, the antimicrobial compounds pro-duced by our strains were proteinous, heat-stable and had a bactericidal mode of action, they might satisfy the criteria for bacteriocins (van Laack et al., 1992).

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