PRODUCTION AND CHARACTERIZATION OF BACTERIOCINS PRODUCED BY SOME LAB ISOLATED FROM RAW MILK SAMPLES

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ABSTRACT

Fifty-five strains of lactic acid bacteria (LAB) were isolated from 50 samples of raw milk. The isolates were classified as 32 Lactococcus (19 isolates were identified to species *Lactococcus lactis* subsp. *lactis* and 13 isolates as *Lactococcus lactis* subsp. *cremoris*); while 23 isolates were classified as Lactobacillus (15 isolates were identified to species *Lactobacillus delbrueckii* subsp. *lactis* and 8 isolates as *Lactobacillus acidophilus*). Ten isolates were selected (3 isolates of *Lactococcus lactis* subsp. *lactis*; 2 isolates of *Lactococcus lactis* subsp. *cremoris;* 3 isolates *Lactobacillus delbrueckii* subsp. *lactis* and 2 isolates *Lactobacillus acidophilus*) and employed to produce bacteriocins in different media (milk permeate supplemented MPS, MRS, M17 and TY broth media). The preferable medium for production of bacteriocin extracts was the MPS (conc. 650-750 AU/ml), followed by M17 (conc. 400-550 AU/ml), MRS (conc. 350-500 AU/ml) and TY (conc. 250-450 AU/ml) broth media. All bacteriocin extracts were active against indicator bacteria (G+: *Listeria monocytogenes*, *Staphylococcus aureus* & *Bacillus subtilis* and G-: *Salmonella typhemorium*, *Escherichia coli* & *Pseudomonas fluorescens*). Four extracts were selected from the previous 10 extracts of MPS medium

(which gave the highest inhibition zone against indicator bacteria) to study some characteristics of bacteriocin extracts. They were resistant to heat treatments up to 100ºC for 30 min. and remained full active over a pH range 2 to 8 at 28ºC /16 hr., also they were completely destroyed by pepsin and trypsin, except the bacteriocin extract produced by *Lactococcus lactis* subsp. *lactis*. The activity of bacteriocin extracts was persisted for 90 days at -15ºC but they were stable at ~5ºC until 30 days and gradually decreased throughout 60 to 90 days from storage at ~5ºC. The activity of bacteriocin extracts was gradually decreased during storage at ~25ºC until 60 days and completely lost up to 90 days of storage.

Keywords: bacteriocin extracts, lactic acid bacteria (LAB), storage

INTRODUCTION

Lactic acid bacteria (LAB) are industrially important organisms as recognized for their fermentative ability as their health and nutritional benefits. These unique microorganisms are a part of the daily diet of virtually people allover as indigenous contaminants in raw milk (Du Toit *et al*., 2000; Lee & Paik, 2001 and El-Attar *et al*., 2004). The preservative action of LAB in foods results from the formation of metabolites' with antimicrobial activity, e.g. bacteriocins or bactericidal proteins during lactic fermentations, which make them useful in food biopreservation (Oyetayo *et al*., 2003).

The isolation and characterization of LAB from raw milk samples has been considered the search for new industrial important cultures (El-Soda *et al*., 2003).

Bacteriocins are antimicrobial proteinaceous compounds or small proteins which inhibit, by a bactericidal or bacteriostatic mode of action.

Bacteriocins are inhibitory towards sensitive strains and are produced by both Gram-positive and Gram-negative bacteria (Tagg *et al*., 1976 and De Vuyst & Vandamme, 1994a). Bacteriocins are produced by some strains of LAB; which exhibit antimicrobial activity against strains closely related to the producer microorganisms (Du Toit *et al.*, 2000). Most of the bacteriocins produced by LAB are active against Gram-positive bacteria including *Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis* & *Clostridium spp*., and a wide range of Gram negative bacteria including *Pseudomonas sp.*, *Salmonella sp.*, *Escherichia coli*, (Carrasco *et al.*, 2002).

Several studies compared bacteriocins produced by LAB strains on different complex media and found that MRS and Elliker broths were the best media for this production (Daba *et al*., 1993). However, such media are too expensive for an economical production process. Recently, there has been increasing interest in producing bacteriocin from permeate since certain LAB strains can grow and produce appreciable amounts of bacteriocin in this low-cost medium (Daba *et al*., 1993 and De Vuyst & Vandamme, 1994a).

The present study was planned to isolate LAB from raw milk samples able to produce high active amount of bacteriocin and then, study the bacteriocin characterization.

MATERIALS AND METHODS

Isolation and selection of LAB strains:

Fifty samples of fresh milk were collected from the local farms and laboratories in Khaliobeia and Cairo Gavernorates under aseptic conditions to isolate LAB producing bacteriocin. One ml milk was diluted in 9 ml sterilized saline solution. Appropriate dilutions were spread on Elliker,

De, Man Rogosa and Sharp (MRS) and Molten 17 (M17) agar media. The plates were incubated at 32°C for 72 hr under aerobic condition and the isolates were purified by dilution technique and streaking two times on the same media.

140 pure isolates (took numbers from 1 to 140) were randomly picked up from suitable plates of Elliker, MRS and M17 agar media and each isolate was tested on Elliker broth after incubation at 32°C for 48 hr to isolate lactic acid bacteria strains by microscopic examinations (cell morphology according to their shape and colony, then they examined by gram stain reaction and endospore stain), tested for catalase production, facultative anaerobic or microaerophilic, aerobic of obligate (Marschall, 1993 and Bergey's Mannual, 1994) and coagulation of milk (El Attar *et al*., 2004).

Classification and identification of LAB isolates:

Isolated strains of LAB were classified and identified according to Bergey's Mannual (1994) by biochemical, physiological tests and microscopic examinations.

Activation of LAB isolated strains:

Ten isolates of LAB were selected (3 isolates of *Lactococcus lactis subsp. lactis*; 2 isolates of *Lactococcus lactis subsp. cremoris*; 3 isolates *Lactobacillus delbrueckii subsp. lactis* and 2 isolates *Lactobacillus acidophilus*) and activated in M17 broth at 30°C/24 hr for Lactococcus and MRS broth at 37°C/24 hr for Lactobacillus. The isolates were reactivated twice with successive transfers in the previous media (10^6-10^7cfu ml^{-1}) and then kept in refrigerator until use, through 24 hr (Abd El-Fattah, 1999).

Activation of spoilage and pathogenic strains:

Spoilage strains [*Bacillus subtilis* 14308 (*B. subtilis*), *Escherichia coli* 0157:H_7 (*E.coli*) and *Pseudomonas fluorescens* 2F4 (*Pseu. fluorescens*)] and pathogenic strains [*Listeria monocytogenes* 1514 (*List. monocytogenes*)**,** *Staphylococcus aureus* ATCC 14458 (*Staph. aureus*) and *Salmonella typhimurium* ATCC 27853 (*Salm. typhimurium*)] were obtained from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt

Both of spoilage and pathogenic strains were activated on Tryptone soya broth (TSB) at 37°C/24 hr for all strains except, *Psedomonas fluorescens* as it was incubated at 30°C. These strains were reactivated twice (10^6 cfu ml^{-1}) and conserved in refrigerator (Abd El-Fattah, 1999).

Bacteriocins production in different media:

Ten ml from each activated culture of isolated LAB inoculated into one liter of MRS, M17, TY (Tryptone yeast extract) and MPS (Milk permeate supplemented) broth media (pH 6.8) under aseptic conditions and incubated at 30 and 37°C/16 hr for Lactococcus and Lactobacillus isolates respectively as described by Hurst (1966) and Abd El-Fattah (1999).

Milk permeate supplemented broth medium (MPS) was prepared with 2.00% yeast extract, 636 mg/l NH_4Cl, 0.03-0.05% valine, 0.006% pantothenic acid, 2.00% sodium β-glycerophosphate and 1.00 ml tween 80. The pH was adjusted to 6.9 then autoclave sterilized at 121°C/15 min. This medium was prepared according to Abd El-Fattah (1999).

Extraction of bacteriocins:

All bacteriocin produced cultures were adjusted to pH 2.0 with HCl 1N. NaCl 1M was added at level of 10% (v/v), then, all cultures were heated in water bath at 100°C for 5 min. The cells were harvested by

centrifugation at 10,000 rpm for 20 min at 4°C. Pellets were washed by adding a solution of NaCl 1 M at pH 2.0 and re-centrifuged again under the same conditions. Pellets were rewashed twice. The gained supernatants, those actually bacteriocin containing extracts, were collected for every strain (Hurst, 1966; Yang *et al*., 1992 and Savadogo *et al*., 2004).

Bacteriocin activity:

Bacteriocin activity was assayed by the agar-well diffusion method of Tagg and Mc Given (1971), with some modifications (Tahara and Kanatani, 1996). Portion (100 µl) of serial twofold dilutions of supernatant (bacteriocin extract) for every isolated strain were placed into each 1-cm-diameter well of a plate containing Tryptone soya agar medium (TSA), which was inoculated with approximately $1x10^6$ cfu ml^{-1} of log-phase culture of indicator bacterial strains. After aerobic incubation for 16 hr at 30°C of *Pseudomonas fluorescens* and 37°C of all other bacterial strains indicator, plates were examined for inhibition of the indicator lawn. The titre was defined as the reciprocal of the highest dilution showing definite inhibition of the indicator lawn and was expressed in activity units (AU) per ml. Bacteriocin activity was recorded as positive if the width of the clear zone around the colonies of the producer was 2 mm or larger. Inhibition was always observed as a well- defined zone of clearing around the well containing the culture supernatant.

Characterization of bacteriocin extracts:

Sensitivity of bacteriocin extracts to heat treatments, pH values, storage at different temperatures and enzymes (Lyophilized crystalline Pepsin 300 U/mg and Trypsin 2000 U/G enzymes were obtained from Laboratories chemicals and reagents, LTD. Netherlands) were measured by well agar diffusion assay:

The sensitivity of bacteriocin extracts to heat treatments were tested by heating cell free supernatant (5 ml) to 40, 60, 80 and 100°C / 30 min, or autoclaved at 121°C / 15 min. The activity was tested by the agar-well diffusion assay (Ivanova *et al*., 2000 and Lee and Paik, 2001). The bacteriocin sensitivity to pH values was tested as described by (Kelly *et al*., 1996). The supernatant fluid of bacteriocin extract was adjusted to pHs 2, 4, 6, 8, 10 and 12 with NaOH 1N or HCl 1 N and incubated at 28°C for 16 hr. The control treatment was adjusted to pH 6 and then tested without incubation. Bacteriocin activity was tested by the agar-well diffusion assay after readjusting pH 6 to exclude antimicrobial effect of organic acid.

Effect of proteolytic enzymes on the bacteriocin activity was tested according to Kelly *et al*., 1996. The bacteriocin supernatant fluid was treated with 1.0 mg ml^{-1} trypsin at pH 7 or pepsin at pH 3 for 1 hr/37°C and then readjusting pH to 6. The mixture was heated at 100°C for 10 min to denature the enzymes (Park *et al*., 2003). Bacteriocin activity was measured by the agar-well diffusion method.

To evaluate the stability over time, bacteriocin extract was stored at room temperature (~25°C)**,** refrigerating (~5°C) and freezing (-15°C) for 90 days and activity was evaluated every 10 days. Bacteriocin activity was assayed by the agar-well diffusion method (Corsetti *et al*., 2004).

Statistical analysis:

Statistical analysis for the obtained data was carried out according to the methods described by Clarke and Kempson (1997).

RESULTS AND DISCUSSION

Isolation, classification and identification of LAB from raw milk samples

Diagram (1) shows the results of isolation, classification and identification of LAB strains from 140 isolated bacteria picked up from 50 milk samples using biochemical, physiological tests and microscopic examinations described according to Marschall (1993); Bergey's manual (1994) and El Attar *et al*. (2004). The results revealed that 19 isolates were identified as *Lc. lactis subsp. lactis*, 13 as *Lc. lactis subsp. cremoris*, 8 as *Lb. acidophilus* and 15 as *Lb. delbrueckii subsp. Lactis*.

Production of bacteriocins by the isolated LAB from raw milk samples on different media

Table (1) illustrates the activity of bacteriocins extracted from ten isolates of LAB which were selected to produce bacteriocins (*Lactococcus lactis* subsp. *lactis* No.8, 20 & 30; *Lactococcus lactis* subsp. *cremoris* No. 36 & 56; *Lactobacillus acidophilus* No. 77 & 87 and *Lactobacillus delbrueckii* subsp. *lactis* No. 94, 95 & 97) growning on different broth media (MPS, MRS, M17 and TY). Bacteriocin activity was determined by the well agar diffusion using *Listeria monocytogenes* as indicator.

The results clear that, bacteriocin produced by all strains recorded its maximum activity (conc. 650 -750 AU/ml) in MPS, followed by M17 (conc. 400-550 AU/ml), MRS (conc. 350-500 AU/ml) and lastly TY (conc. 250-450 AU/ml) broth media. *Lactococcus lactis* subsp. *lactis* gave the highest production of bacteriocin activity. Variations between treatments with bacteriocins extracted from MPS medium and the other treatments were significant ($P \leq 0.05$).

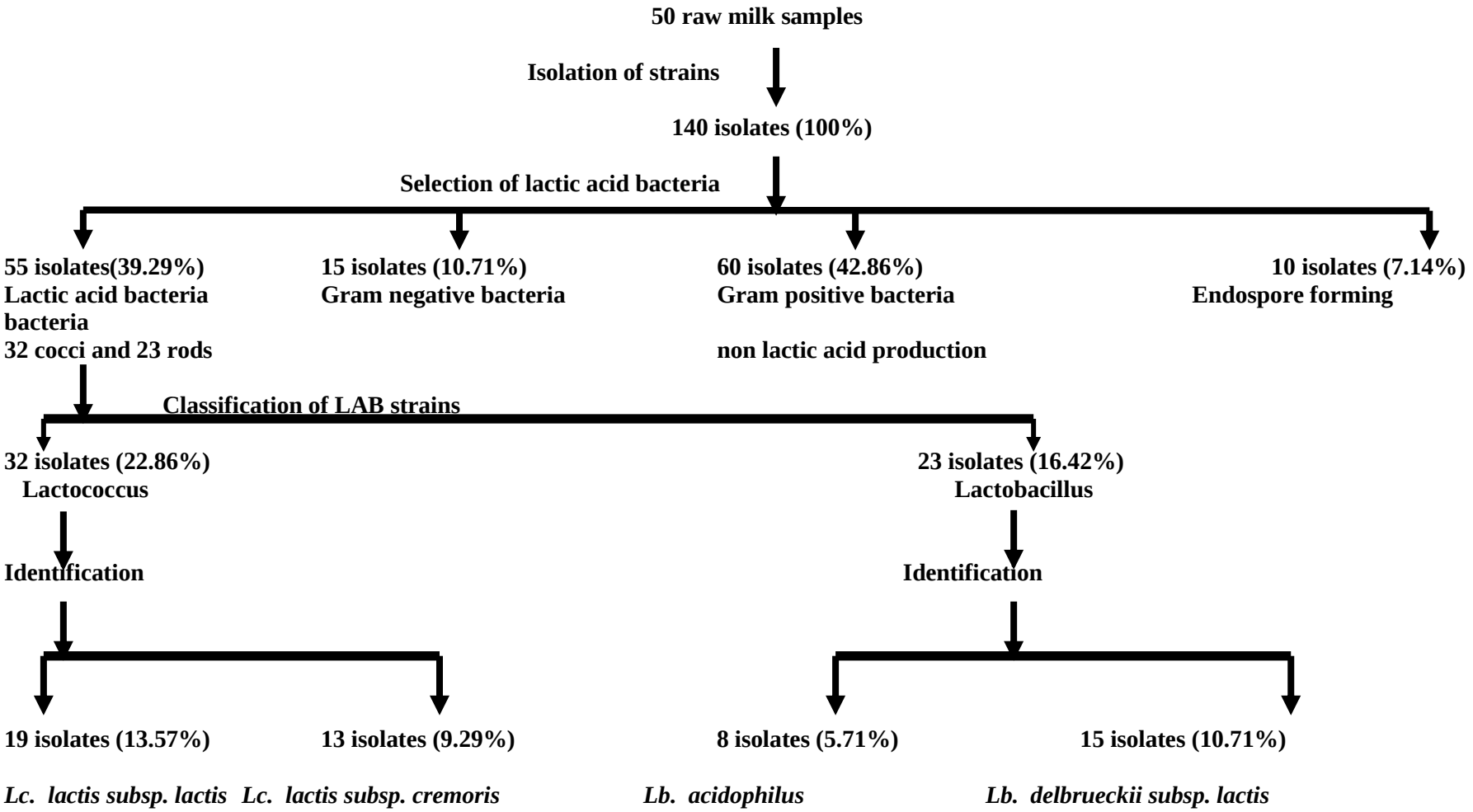

Diagram (1): Results of isolated, classified and identified LAB strains from raw milk samples.

Table (1): Activity of bacteriocins extracted from Lactococcus and Lactobacillus grown on different broth media using the well agar diffusion.

Production media / Selected strains		Activity of bacteriocin (AU/ml)			
		MPS	**M17**	**MRS**	**TY**
Lc. lactis subsp. *lactis*	**No.8**	750	550	500	450
	No.20	700	450	400	300
	No.30	650	400	350	350
Lc. lactis subsp. *cremoris*	**No.36**	650	450	400	350
	No.56	650	550	500	400
Lb. acidophilus	**No. 77**	700	400	450	300
	No. 87	700	450	500	350
Lb. delbrueckii subsp. *lactis*	**No. 94**	700	450	500	400
	No. 95	650	400	500	250
	No.97	700	400	450	400

MPS = Milk permeate supplemented medium
MRS = De Man, Rogosa and Sharp medium
M17 = Molten 17 medium
TY = Tryptone yeast extract medium

Similar results were obtained by Morgan *et al*. (1999) who found that the production of lacticin activity by *Lactococcus lactis subsp. lactis* DPC3147 was high in almost all dairy based media; while, lower levels lacticin activity were observed in TY broth medium. Moreover, Abd El-Fattah, (1999) reported that, the utilization of *Lactococcus lactis* to produce antibacterial substances (ABS) in permeate supplemented with some additives, led to the highest amount of ABS.

Since, MPS is readily available and good for bacteriocins production, it could be the preferable medium.

- The inhibition effect of extracted bacteriocins:

Table (2) shows the inhibition zone diameters as a result of the effect of extracted bacteriocins (from MPS, MRS, M17 and TY broth media) on growth of various indicator bacteria (gram positive, e.g. *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis*, and gram

Table (2):The inhibition effect of extracted bacteriocins (from different media) on growth of some indicator bacteria (Gram^{+}& Gram $^{-}$) grown in T.S.A medium.

Selected strains		Diameter of inhibition zone (mm)											
		Listeria monocytogenes (G^{+})				*Staphylococcus aureus* (G^{+})				*Bacillus subtilis* (G^{+})			
		MPS	**M17**	**MRS**	**TY**	**MPS**	**M17**	**MRS**	**TY**	**MPS**	**M17**	**MRS**	**TY**
Lc. lactis* subsp. *lactis	No. 8	13.33	11.66	9.66	9.33	14.00	11.66	10.00	9.00	13.00	11.66	10.00	7.66
	No. 20	13.00	11.50	10.00	9.00	12.66	12.00	9.50	8.66	12.33	11.00	10.00	8.00
	No. 30	12.00	11.33	11.00	8.66	13.00	11.50	10.66	8.50	11.66	10.50	9.66	7.33
Lc. lactis* subsp. *cremoris	No. 36	11.66	10.33	9.00	8.00	11.66	10.66	9.33	7.66	11.66	10.66	9.66	8.00
	No. 56	12.00	11.0	9.33	8.66	12.33	11.33	10.00	8.66	12.00	10.50	10.00	7.66
Lb. acidophilus	No. 77	12.33	9.66	11.33	8.66	12.33	9.66	11.33	8.66	12.33	9.66	11.33	8.00
	No. 87	13.00	10.33	11.50	8.33	12.66	10.00	11.50	8.00	12.66	9.33	11.00	7.33
Lb. delbrueckii* subsp. *lactis	No. 94	11.66	9.66	10.50	8.33	11.50	9.50	10.66	8.33	11.50	9.50	10.66	7.66
	No. 95	11.33	9.33	10.33	7.66	11.33	9.33	10.33	8.00	11.33	9.33	10.33	7.50
	No. 97	11.00	9.50	10.33	8.00	11.00	9.00	10.50	7.66	11.00	9.00	10.50	7.33
		Salmonella typhimurium (G^{-})				*Pseudomonas flourescens* (G^{-})				*Escherichia coli* (G^{-})			
Lc. lactis* subsp. *lactis	**No.8**	11.50	11.00	9.66	8.00	12.00	10.66	9.33	8.00	12.00	10.66	9.33	8.33
	No.20	11.33	10.33	9.33	7.66	11.00	10.50	9.66	7.33	11.66	10.33	9.00	8.00
	No.30	10.66	10.00	9.50	8.00	11.33	10.00	9.00	7.50	10.66	10.00	9.66	7.50
Lc. lactis* subsp. *cremoris	**No. 36**	11.00	10.33	9.33	7.33	10.66	10.00	9.00	7.33	10.33	9.66	8.66	7.33
	No. 56	11.33	10.50	9.50	8.00	11.00	10.33	9.66	7.66	11.00	10.00	9.33	8.00
Lb. acidophilus	**No. 77**	10.66	9.00	10.00	7.50	11.00	9.00	10.00	7.33	11.33	9.00	10.00	7.33
	No. 87	11.00	9.33	10.33	7.66	11.66	9.50	10.66	7.66	12.33	9.66	10.66	7.66
Lb. delbrueckii* subsp. *lactis	**No. 94**	10.66	9.00	10.00	8.00	11.00	9.00	10.00	8.00	11.33	9.50	10.50	7.66
	No. 95	10.33	9.00	10.00	7.33	10.33	9.00	9.66	8.33	10.33	9.00	10.33	7.50
	No. 97	10.50	8.66	9.66	7.33	10.50	8.66	10.00	7.33	11.00	9.33	10.00	7.00

MPS = Bacteriocin extract from milk permeate supplemented medium. **MRS = Bacteriocin extract from MRS medium**

TY = Bacteriocin extract from tryptone yeast extract broth medium. **M17 =Bacteriocin extract from M17 medium**

negative, e.g. *Salmonella typhimurium, Pseudomonas fluorescens and Escherichia coli*) grown in TSA medium.

The highest antibacterial activity of extracted bacteriocins from MPS broth medium against the indicator bacteria were significant ($P \leq 0.05$) at TSA medium. While, the extracted bacteriocins from TY broth medium gave the lowest inhibition values ($P \leq 0.05$).

The results of the inhibition effect of extracted bacteriocins revealed that, the inhibition effect was significantly ($P \leq 0.01$) higher on gram positive indicator bacteria than that of gram negative indicator bacteria. This may be due to that the gram positive bacteria are much more sensitive to the bacteriocin produced by LAB than gram negative bacteria. The variations of bacteriocin sensibility are due to the characteristics of indicator strains (presence or absence of receiving sites or immunoprotein). The resistance of gram negative bacteria are attributed to the particular nature of their cellular envelope, the mechanisms of action described for bacteriocins bringing in a phenomenon of adsorption (Sanni *et al.*,1999).

Characterization of bacteriocin extracts

Four bacteriocin extracts were selected from the previous 10 extracts produced by LAB strains which gave the higher inhibition zones (bacteriocin extracts produced from *Lactococcus lactis* subsp. *Lactis* No.8, *Lactococcus lactis* subsp. *cremoris* No. 56, *Lactobacillus acidophilus* No. 78 and *Lactobacillus delbrueckii* subsp. *lactis* No. 94) to study some bacteriocin characteristics.

Heat treatments

Fig.(1A) reveals that all bacteriocin extracts were stable to heat treatments up to 80ºC for 30 min against indicator bacteria. While, there was very slight reduction of inhibition zone for the same indicator bacteria by raising heat treatment to 100ºC for 30 min, the inhibition activity of all bacteriocin extracts was completely inactivated by heat treatment at 121ºC for 15 min (Sterilization). The differences between heat treatments up to 100ºC and 121ºC were significant ($P \leq 0.05$). This heat stability could be due to the formation of small globular structures and the occurrence of strongly hydrophobic regions, stable cross-linkages, and a high glycine content (De Vuyst and Vandamme, 1994b).

Similar results were observed by Card inal *et al*. (1997); Lee & Paik (2001); Carrasco *et al*. (2002) and Ayad, (2004) who found that the inhibitory activities of bacteriocins produced by *Lactococcus lactis* subsp. *Lactis*; *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus acidophilus* strains were detected after heat treatments up to 100ºC for 30 min.

PH values

The sensitivity of the inhibitory bacteriocin extracts to different pH values (2, 4, 6, 8, 10 and 12) with incubation at 16 hr. compared with control at pH 6 without incubation, was shown in Fig. (1B). It was observed that, bacteriocin extracts were very stable over a wide pH range of 2 to 6. While the activity significantly decreased by increasing the pH to 8 and 10, the activity of bacteriocin extracts were completely inactivated at pH 12 ($P \leq 0.05$). Similar results were obtained by Cardinal *et al*.(1997) who stated that, bacteriocins retained their activity from pH 3 to 5.

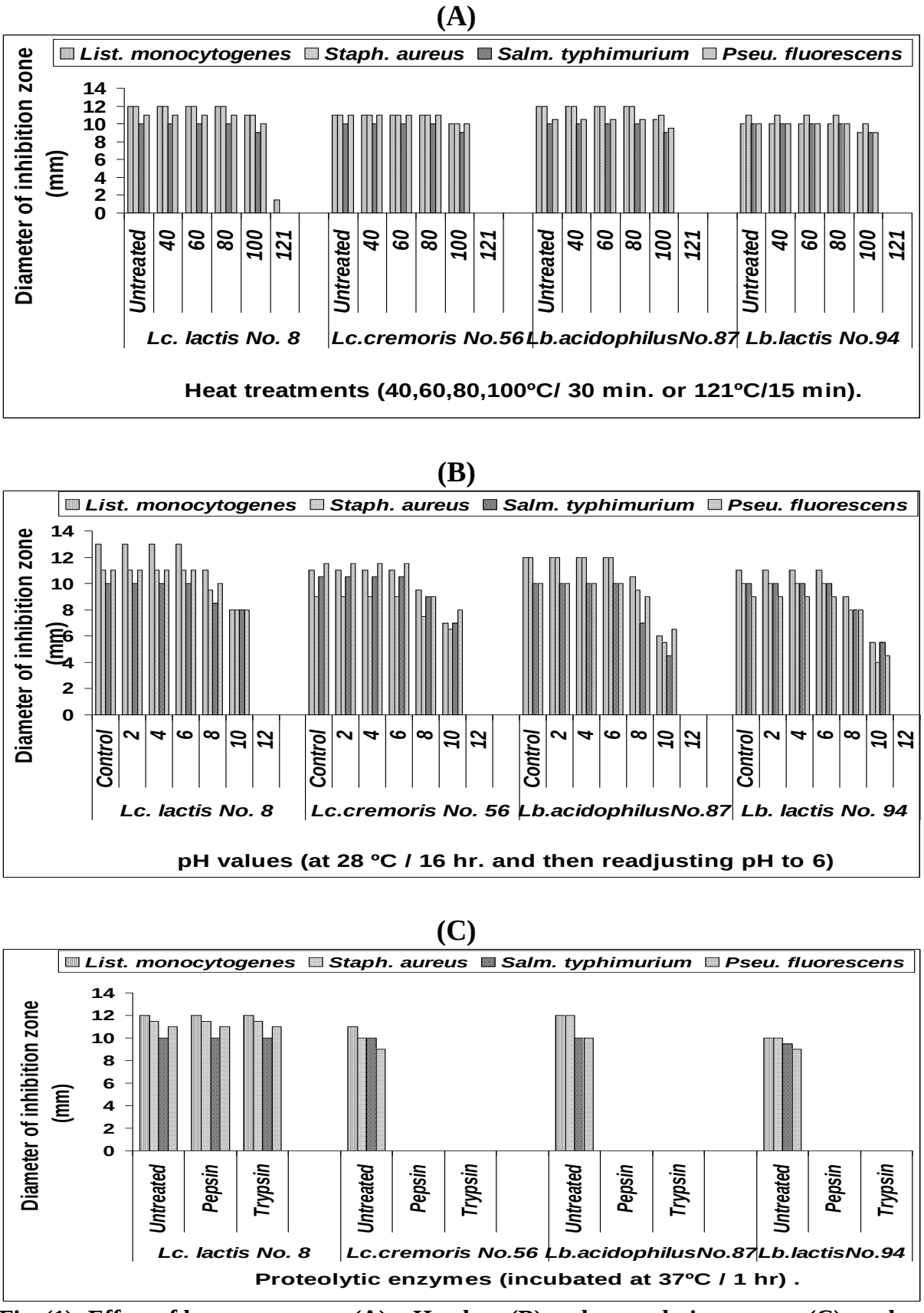

Fig. (1): Effect of heat treatments (A), pH values (B) and proteolytic enzymes (C) on the stability of bacteriocin extracts produced by selected LAB.

Proteolytic enzymes

Data in Fig. (1C) reveals that the inhibition zone for bacteriocin extract produced from *Lactococcus lactis* subsp. *lactis* No. 8 against the indicator bacteria was not sensitive to pepsin and trypsin as it remained active. These results are in accordance with that obtained by Ivanova *et al*. (2000); Lee & Paik (2001) and Ayad (2004).The antibacterial activity of bacteriocin extracts produced from *Lactococcus lactis* subsp. *cremoris* No. 56, *Lactobacillus acidophilus* No. 78 and *Lactobacillus delbrueckii* subsp. *lactis* No. 94 were completely inhibited by treatment with pepsin and trypsin as they gave no zones compared with untreated bacteriocin extract.

Tahara &Kanatani (1996); Carrasco *et al*. (2002) and Savadogo *et al*. (2004) studied the effect of enzymes on the antimicrobial activities of *Lactococcus lactis* subsp. *cremoris*, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis*. They found that the activities of the bacteriocins were lost after treatment with pepsin and trypsin.

Storage on different temperatures

Fig. (2) shows the effect of storage at room temperature (~25), ~5 and -15ºC for duration of three months on stability of bacteriocin extracts.

The activity of bacteriocin extracts was significantly ($P \leq 0.05$) decreased by storage progress. After 90 days of storage, the activity of bacteriocin extracts completely lost at ~25ºC. Bacteriocins were stable during storage at ~5ºC until 30 days, then activities were significantly ($P \leq 0.05$) decreased after 60 to 90 days of storage. Moreover, the activity of bacteriocin extracts was persisted for 90 days of storage at -15ºC. Statistical analysis data for storage at -15 ºC show insignificant difference for all treatments.

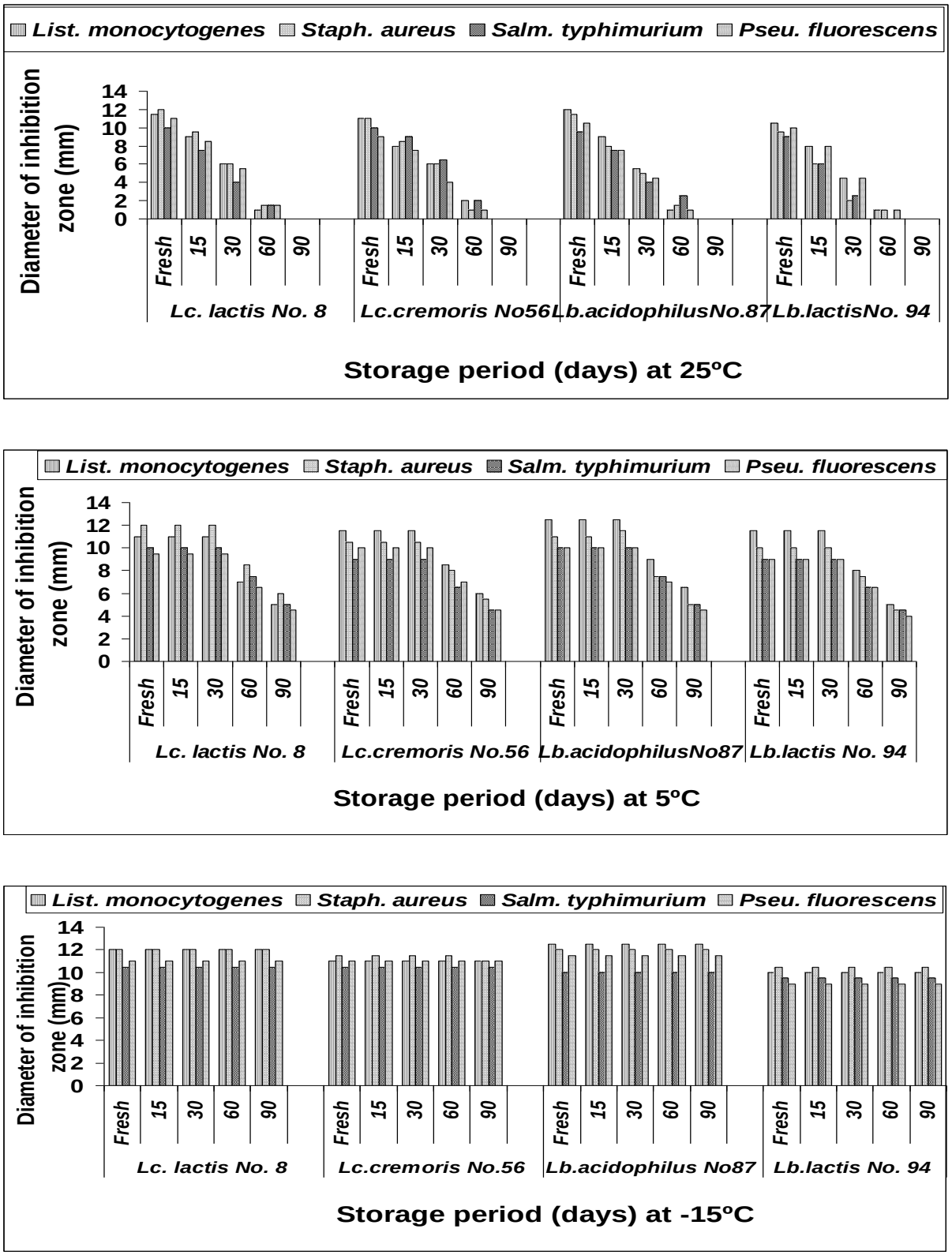

Fig.(2):Effect of storage at different temperature on the stability of bacteriocin extracts produced by selected LAB.

Carrasco *et al*. (2002); Corsetti *et al*. (2004) and Ivanova *et al*. (2000) found that, the bacteriocin activity produced by Lactococcus spp. and Lactobacillus spp. were partially or completely lost at 25ºC and stable at 4ºC and -20ºC during storage for at least 3 months.

From the foregoing results, it can be recommend that the preferable medium for production of bacteriocin from LAB was the milk permeate supplemented (MPS).

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إنتاج وخصائص البكتريوسينات المنتجة بواسطة بعض سلالات بكتريا حامض اللاكتيك المعزولة من عينات لبن خام

تم عزل ٥٥ سلالة لبكتريا حامض اللاكتيك من ٥٠ عينة لبن خام. ثم صنفت إلي ٣٢ عزلة تنتمي لجنس Lactococcus (١٩ عزلة عرفت بـ *Lactococcus lactis* subsp. *Lactis* و ١٣ عزلة عرفت بـ *Lactococcus lactis* subsp. *cremoris*) و ٢٣ عزلة تنتمي لجنس Lactobacillus (١٥ عزلة عرفت بـ *Lactobacillus delbrueckii* subsp. *Lactis* و ٨ عزلات عرفت بـ *Lactobacillus acidophilus*). تم اختيار ١٠ سلالات (٣ سلالات *Lactococcus lactis* subsp. *Lactis* و ٢ سلالة *Lactococcus lactis* subsp. *Cremoris* و ٣ سلالات *Lactobacillus delbrueckii* subsp. *Lactis* و ٢ سلالة *Lactobacillus acidophilus*) واختبرت لإنتاج البكتريوسينات علي البيئات السائلة المختلفة (بيئة يرميت اللبن المدعمة MPS و MRS و M17 والتربتون ومستخلص الخميرة TY). وكانت أفضل بيئة لإنتاج مستخلص البكتريوسينات هي MPS (بتركيز ٦٥٠- ٧٥٠ وحدة نشاط / مل) يليها M17 (بتركيز ٤٠٠- ٥٥٠ وحدة نشاط / مل) ثم MRS (بتركيز ٣٥٠- ٥٠٠ وحدة نشاط / مل) و TY (بتركيز ٢٥٠- ٤٥٠ وحدة نشاط / مل). ووجد أن كل مستخلصات البكتريوسين كانت نشطة ضد البكتريا المرضية والمسببة للفساد (الموجبة لجرام *Listeria monocytogenes, Staphylococcus aureus* & *Bacillus subtilis* والسالبة لجرام *Salmonella typhemorium*, & *Pseudomonas fluorescens* , *Escherichia coli*).

تم اختيار ٤ مستخلصات بكتريوسين من الـ ١٠ مستخلصات السابقين لبيئة MPS (التي أعطت أعلي نشاط ضد البكتريا المرضية والمسببة للفساد) لدراسة خصائصها. وجد أن مستخلصات البكتريوسين قاومت الحرارة إلي ١٠٠°م /٣٠ ق ، كما ظلت مستخلصات البكتريوسين نشطة علي pH من ٢ إلي ٨ / ٢٨°م /١٦ ساعة ، وعند معاملة مستخلصات البكتريوسين بالببسين والتربسين حدث فقد تام في نشاطها ماعدا مستخلص البكتريوسين المنتج بواسطة *Lactococcus lactis subsp. Lactis* No.8 لم يتأثر. كما لم يحدث تغير في نشاط مستخلصات البكتريوسين عند تخزينها لمدة ٩٠ يوم / - ١٥ °م ، كما ظلت مستخلصات البكتريوسين ثابتة عند التخزين علي ~ ٥°م لمدة ٣٠يوم ثم حدث انخفاض تدريجي خلال ٦٠ الي ٩٠ يوم. بينما انخفض نشاط مستخلصات البكتريوسين تدريجيا أثناء التخزين علي ~ ٢٥°م حتى ٦٠ يوم ثم حدث فقد تام في النشاط عند ٩٠ يوم من التخزين.