Effect of Bacteriocin Extracted from *Lactobacillus acidophilus* on the Shelf-life of Pasteurized Milk

*Ekbal M. A. Ibrahim and Hend A. Elbarbary

Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt
*ekbal_adel_2200 @yahoo.com

Abstract: Bacteriocins are natural metabolites produced by many strains of Lactic acid bacteria that used in food bioprocessing. They have potential healthy role in suppressing the growth of some spoilage and pathogenic bacteria. Bacteriocin from *Lactobacillus acidophilus* strain was extracted and inoculated into freshly pasteurized milk at concentrations of 320, 160, 80 & 40 IU/mL, then the milk samples were examined for T.A% & microbiological examination at the time of preparation and at 3 days intervals till sings of spoilage were detected. Obtained results revealed that pasteurized milk samples with bacteriocin at concentration 160 and 320 IU/mL showed the lowest T.A% & highly significant inhibitory effect on total bacterial count, aerobic spore formers and psychrotrophic count, as well as it could extend the shelf-life of pasteurized milk up to 12 days during refrigerator storage. While, for those samples with 80 IU/mL showed no effect on both total bacterial count & psychrotrophic count with slight reduction for aerobic spore formers. While, those with 40 IU/mL & control ones (without bacteriocin) could not control the bacterial contaminant counts and the signs of spoilage appeared at the 9th day of storage.

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1. Introduction

Bacteriocins are ribosomally-synthesized peptides or proteins with antimicrobial activity against many food-borne pathogens and spoilage bacteria in food (**Muñoz *et al*., 2007**). They are produced by different strains of Lactic acid bacteria such as Lactococcus, Pediococcus, Leuconostoc, Enterococcus and Lactobacillus (**Mc Auliffe *et al*., 2001**). *Lactobacilus acidophilus* is the most important LAB used for production of bacteriocin (**BogovicMatijasic et al., 1998**). Bacteriocins have several potential characteristics rendering them useful natural food preservatives as they are small hydrophobic cationic peptides, stable at a wide range of pH and temperature and considered as safe (GRAS) (**Cleveland et al., 2001**). In addition to being non-toxic to eukaryotic cells; they are effectively therapeutics as they are typically more potent than conventional antibiotics with different spectra of activity as they differ from most therapeutic antibiotics in being proteineous agents that are rapidly digested by proteases in human digestive tract and have an important role in reducing the incidence of diarrhea (**Rossi et al., 2008** and **Saavedra et al., 2004**).

Bacteriocins are used as a tool to control the growth of undesirable microbial growth including spoilage and pathogenic bacteria and to keep the food more acceptable to consumer (**Deegan et al., 2006**).

On the other hand, some contaminants like aerobic spore formers and psychrotrophic microorganisms may multiply in food during its storage, thus affecting its safety and quality as pasteurized milk leads to spoilage problems as bitty cream, off-flavors or sweet curdling ,decrease its shelf-life and substantial economic losses (**Reij et al., 2004**).

Pasteurized milk shelf-life is a responsibility shared by producers, processors, retailers and consumers and owing to relatively short shelf-life which not exceed one week at refrigerator temperature. This study was planned to evaluate the effect of bacteriocin extracted from *L. acidophilus* on the quality of pasteurized milk and its role to extend its shelf-life.

2. Materials and methods

2.1. Activation of *Lactobacillus acidophilus*

Lyophilized strain of *L. acidophilus* DSMZ 20079 was obtained from Cairo MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. *Lactobacillus acidophilus* strain was activated on MRS broth (De Man, Rogosa and Sharp which obtained from Biolife, Italy) at 37 ℃ for 24hrs then three transfers were performed to activate this culture. Serial dilutions were prepared till obtaining the concentration of 10^7- 10^9 CFU/mL in order to meet the required recommended level for active probiotic (**IDF, 1988**). Then, 10 mL of activated culture of *L. acidophilus* was separately inoculated into one liter of MRS broth under aseptic conditions

and incubated at 37℃ for 16 hrs as described by **Chumchalova et al. (2004).**

2.2. Extraction of bacteriocin

Bacteriocin producing culture was adjusted to pH 2.0 by adding HCL 1N then culture was heated in a water bath at 100℃ for 5 min. The cells were harvested by centrifugation at 10,000 rpm for 20 min at 4℃. The cell-free supernatant containing bacteriocin extract was adjusted to pH 6.0 using NaOH 1N to exclude the effect of organic acids 1N. Then bacteriocin extract was sterilized by using Seitz filter to eliminate the possible presence of viable cells (**Simova et al., 2009**).

2.3. Detection of bacteriocin titer

The titer of bacteriocin was quantified by agar well diffusion method according to **Todorov and Dicks** (**2004**). Indicator strains at concentration of about 10^7 CFU/mL was inoculated into Muller Hinton agar, poured into petri dishes and allowed to solidify. Wells were made on the solidified agar with sterile cork borer (10 mm in diameter) then inoculated with 100 µL of two fold serial dilutions from bacteriocin extract. The plates were incubated at 37 °C for 24hrs and then examined for the presence of clear zone of inhibition (2 mm or wider) around the wells. The titer of inhibition was defined as the reciprocal of the highest dilution showed definite inhibition zone and was expressed

The activity unit (AU/mL) was calculated according to the following formula:

$$\mathbf{AU= (1000/V) \times 2^{y}}$$

Where, **AU** is arbitrary Unit of bacteriocin activity, **y** is the number of the last dilution showed inhibition, and **V** is the volume of supernatant (µL) which inoculated in each well.

2.4. Preparation of pasteurized milk samples

Five flasks (250 mL screw-cap flask), each containing 200 mL raw milk samples , were subjected to low temperature short time pasteurization at 65°C for 30 min. The pasteurized milk of the first flask was inoculated with bacteriocin at concentration 320 IU/mL (T1), the second with 160 IU/mL (T2), the third with 80 IU/mL (T3), the fourth with 40 IU/mL (T4), while the last one is the control which contains no bacteriocin (T5). Each treated flask was kept at refrigerator and the samples were examined for acidity and bacteriologically at zero time, then at three days intervals till the signs of spoilage were detected. Each treatment was triplicate.

2.5. Examination of prepared samples

2.5.1. Titratible acidity (T.A) measurement

The T.A% was detected according to **Pearson** (**1984**).

2.5.2. Bacteriological examination

Pasteurized milk samples were prepared for tenth fold serial dilution, and then plate count agar plates were used to enumerate the total bacterial count (TBC) and psychrotrophs at 37°C for 24-48 h and 7°C for 7-10 days, respectively. Dextrose trypton agar medium (DTA) was used for aerobic spore formers at 37°C for 24-48 h (**APHA, 1992**).

2.6. Statistical analysis

Statistical comparisons were made by using one-way analysis of variance (ANOVA). The results were considered significantly different with $P < 0.05$ as described by **Clarke and Kempson (1997)**.

3. Results and Discussion

There is an increasing interest in the research of antimicrobial peptides, bacteriocins and bacteriocins-like compounds produced by LAB because of their potential use as natural preservatives for improving the safety of food. Bacteriocins are generally of low molecular weight proteins with bactericidal effect on Gram-positive bacteria and bacteriostatic action on Gram-negative bacteria (**O'Sullivan et al., 2002**).

3.1. Determination of titratable acidity (T.A)

Samples of pasteurized milk were firstly tested to evaluate the percentage of lactic acid during storage period as titratable acidity acts as a good indicator of its quality. There was a slight gradual increase of T.A% with progressive storage period extended up to 12 days for pasteurized milk samples treated with bacteriocin at concentration 320 IU/mL (T1) and 160 IU/mL (T2) with mean values of 0.17 ± 0.01 and 0.19 ± 0.02; respectively . On the other hand, the mean T.A% for samples treated with bacteriocin at concentration 80 IU/mL (T3) reached 0.18 ± 0.02 % at 9th day and 0.18 ± 0.01 and 0.19 ± 0.01 % at 6th day of storage for 40 IU/mL (T4) and control samples (T5); respectively (**Table 1**). This indicated that the increase of T.A% in T4 and T5 was faster than those of T1 and T2. This would be attributed to the presence of heat resistant lactic acid producing bacteria, particularly streptococci and lactobacilli, which can retain their activity and ferment lactose into lactic acid **(Ruegg and Reineman, 2002)**. While, for T1 and T2, similar effect was found in yoghurt manufactured by bacteriocin extracted from *Lactobacillus acidophilus* that prolonged its coagulation time and this would be attributed to the inhibitory effect of bacteriocin on the lactic acid bacteria and subsequently on their ability to produce acid which leads to slow rate of acid development (**Oh et al., 2006).**

3.2. Effect of bacteriocin on TBC in pasteurized milk

The main objective of milk pasteurization is to eliminate the pathogenic microorganisms and to reduce the initial microbial count to a level safe for human consumption. But, its effectiveness is depend on the type of microorganism present and its concentration **(CFDRA, 1992)**. It is critical to dairy product producers to evaluate the microbial quality of raw milk to ensure that milk is of high quality and its initial microbial count should not exceed 100.000 CFU/mL to meet with the FDA guidelines and as a result produce pasteurized milk with TBC not exceed 20.000 CFU/mL **(U.S. Public Health Service, 1995).**

Table(1): Titratable acidity of pasteurized milk samples with different concentrations of bacteriocin

Storage time	T1	T2	T3	T4	T5
Zero time	0.13 ± 0.01^{A1}	0.13 ± 0.01^{A1}	0.13 ± 0.01^{A1}	0.13 ± 0.01^{A1}	0.13 ± 0.01^{A1}
3rd day	0.12 ± 0.01^{A1}	0.13 ± 0.01^{A1}	0.12 ± 0.01^{A1}	0.17 ± 0.01^{B2}	0.16 ± 0.01^{B2}
6th day	0.12 ± 0.01^{A1}	0.14 ± 0.01^{AB1}	0.14 ± 0.01^{AB1}	0.18 ± 0.01^{B2}	0.19 ± 0.01^{B2}
9th day	0.16 ± 0.01^{A2}	0.18 ± 0.01^{A2}	0.18 ± 0.02^{B2}	(S)	(S)
12th day	0.17 ± 0.01^{A3}	0.19 ± 0.02^{B3}	(S)	(S)	(S)
15th day	(S)	(S)	(S)	(S)	(S)

T1: Pasteurized milk with bacteriocin at concentration 320 IU/ ml
T2: Pasteurized milk with bacteriocin at concentration 160 IU/ ml
T3: Pasteurized milk with bacteriocin at concentration 80 IU /ml
T4: Pasteurized milk with bacteriocin at concentration 40 IU/ ml
T5: Pasteurized milk without bacteriocin (control)
S: Samples showed spoilage
ABC Values in the same row having different superscripts differ significantly ($P < 0.05$).
123Values in the same column having different superscripts differ significantly ($P < 0.05$).
The values indicated were the mean of three trials ± SE (Standard Error).

Many trials were done to control the microbial populations of pasteurized milk. The mean TBC (log $_{10}$CFU/mL) in the examined pasteurized milk samples treated with bacteriocin at concentration 320 IU/mL (T1); 160 IU/mL (T2); 80 IU/mL (T3); 40 IU/mL (T4) and control samples (T5) were 3.00 ± 0.35; 3.13 ± 0.29 ; 3.10 ± 0.23; 3.23 ± 0.05 and 3.00 ± 0.41; respectively at zero time (**Table 2**). Bacteriocin treated milk samples of T1 and T2 showed the lowest TBC that extended their shelf-life up to 12 days with mean values of 2.00 ± 0.40 and 2.93 ± 0.06 log $_{10}$CFU/mL ; respectively. These came in accordance with **EOSQ (2005)** for pasteurized milk in which TBC should not exceed 3.00×10^4 CFU/mL. Bacteriocin extracted from *L. acidophilus* showed significant effect ($P< 0.05$) in reducing the bacterial load of pasteurized milk. The reduction of viable cells in pasteurized milk may be attributed to role of bacteriocin which has synergistic effect with some endogenous components of milk, such as lysozyme and lactoferrin or with some metabolites produced by milk microflora **(Branen & Davidson, 2004 and Nattress et al., 2001)**.

The antimicrobial action of bacteriocins involved increase the interfering with the cell wall of the target cells either by inhibiting cell wall biosynthesis or causing pores leads to the release of the cytoplasmic particles, depolarization of the membrane potential and eventually to cell death (**Ennahar et al., 2000** , **Shai, 2002 and Rossi et al., 2008**).

In this respect, **Bizani et al.** (**2008**) found that addition of 320 IU/mL cerein 8A (bacteriocin extracted from *B. cereus* 8A) to UHT milk resulted in a decrease of three log cycles in viable cells within 14 days at 4°C. As well as, **Sable et al.** (**2000**) reported that bacteriocin at concentration 6.25 µg/mL could destroy 4 log $_{10}$CFU/mL of *E. coli O157: H7* in sterilized skim milk. On the other hand, application of bacteriocin with low concentration showed a relatively no effect on TBC reached up to 5.16 ± 0.30 at 9th day for T3 and 5.00 ± 0.08 and 5.69 ± 0.24 log $_{10}$CFU/mL at 6th day of storage for T4 and T5, respectively (**Table 2**). Defects in milk were detected when microbial concentration reached 5×10^5-10^7 CFU/mL **(Vyletelova et al., 2000)**.

Low temperature short time pasteurization of milk did not significantly affect the number of TBC as they increased from 2.04 log $_{10}$CFU/mL at zero time to 4.77 log $_{10}$CFU/mL at the 10th day of refrigerated storage (**Hassan et al., 2009**). **Korhonen et al. (1998)** stated that heat sterilization of milk is essential to ensure microbial safety and stability.

3.3. Effect of bacteriocin on aerobic spore formers

The aerobic spore formers can contaminate milk supplies from water, udder, teat surfaces, feed, dust

and/or soil, as well as, Milking equipment can also act as reservoirs for the spores into raw milk (**Scheldeman et al., 2002 and Scheldeman et al., 2005**). The most important spoilage bacilli that isolated from UHT milk are *B. licheniformis*, *B. cereus, B. subtilis, B. stearothermophilus* and *B. coagulans* (**Taher, 2004 and Vyletelova et al., 2002**).

The addition of bacteriocin to pasteurized milk of T1 and T2 had significant lower counts of aerobic spore former when compared with control samples (T5). They reached to 1.03 ± 0.03 and 1.30 ± 0.10 log $_{10}$CFU/mL at 12th day of storage; respectively (**Table 3**). In addition, a slightly decrease in the aerobic spore formers was observed from 2.63 ± 0.12 log $_{10}$CFU/mL at zero time to 2.00 ± 0.20 log $_{10}$CFU/mL at day 9 in T3 samples. On the other hand, the aerobic spore former counts of the bacteriocin treated milk samples of T4 were increased 1 log $_{10}$CFU/mL within six days with mean value 3.00 ±0.36 in comparison to 2.5 log $_{10}$ CFU /mL in control samples of T5 with mean value 4.96 ± 0.18 log $_{10}$ CFU /mL (**Table 3**). **Røssland et al. (2003)** found that *L. acidophilus* reduced the *B. cereus* population in sterile milk within 24-72 h, while total inhibition was related to its bacteriocin. In the same respect, viable cell counts of *B. cereus* were reduced by bacteriocin concentrations of 350 IU/g in gelatin pudding after 8 h at 10℃ or after 48 h at 22℃ (**Viedma et al., 2009**).

Table (2): Effect of different concentrations of bacteriocin on TBC (Log$_{10}$ CFU/ ml) in pasteurized milk samples

Storage time	T1	T2	T3	T4	T5
zero time	3.00 ± 0.35^{A3}	3.13 ± 0.29^{AB2}	3.10 ± 0.23^{AB1}	3.23 ± 0.05^{B1}	3.00 ± 0.41^{A1}
3rd day	3.70 ± 0.32^{A3}	3.80 ± 0.76^{A2}	4.00 ± 0.37^{B2}	4.18 ± 0.43^{C2}	4.22 ± 0.31^{C2}
6th day	2.66 ± 0.40^{A2}	3.10 ± 0.47^{B2}	4.50 ± 0.36^{BC3}	5.00 ± 0.08^{BC2}	5.69 ± 0.24^{C3}
9th day	2.86 ± 0.20^{A2}	2.90 ± 0.20^{A1}	5.16 ± 0.30^{B3}	S	S
12th day	2.00 ± 0.40^{A1}	2.93 ± 0.06^{B1}	S	S	S
15th day	S	S	S	S	S

T1: Pasteurized milk with bacteriocin at concentration 320 IU/ ml
T2: Pasteurized milk with bacteriocin at concentration 160 IU /ml
T3: Pasteurized milk with bacteriocin at concentration 80 IU/ ml
T4: Pasteurized milk with bacteriocin at concentration 40 IU/ ml
T5: Pasteurized milk without bacteriocin (control)
S: Samples showed spoilage
ABC Values in the same row having different superscripts differ significantly (P < 0.05).
123 Values in the same column having different superscripts differ significantly (P < 0.05).
The values indicated were the mean of three trials ± SE (Standard Error).

Table (3): Effect of different concentrations of bacteriocin on aerobic spore formers bacteria (Log$_{10}$ CFU/ ml) in pasteurized milk samples

Storage time	T1	T2	T3	T4	T5
zero time	2.06 ± 0.18^{A3}	2.00 ± 0.08^{A2}	2.63 ± 0.12^{B3}	2.00 ± 0.15^{A1}	2.56 +0.14^{B1}
3rd day	2.06 ± 0.08^{A3}	2.53 ± 0.21^{B3}	2.50 ± 0.05^{B3}	2.16 ± 0.18^{A1}	3.80 ± 0.31^{C2}
6th day	1.90 ± 0.10^{A2}	2.06 ± 0.12^{A2}	2.30 ± 0.15^{AB2}	3.00 ± 0.36^{C2}	4.96 ± 0.18^{D3}
9th day	1.00 ± 0.01^{A1}	1.70 ± 0.10^{A1}	2.00 ± 0.20^{B1}	S	S
12th day	1.03 ± 0.03^{A1}	1.30 ± 0.10^{A1}	S	S	S
15th day	S	S	S	S	S

T1: Pasteurized milk with bacteriocin at concentration 320/ IU ml
T2: Pasteurized milk with bacteriocin at concentration 160 IU/ ml
T3: Pasteurized milk with bacteriocin at concentration 80 IU /ml
T4: Pasteurized milk with bacteriocin at concentration 40 IU/ ml
T5: Pasteurized milk without bacteriocin (control)
S: Samples showed spoilage
ABCD Values in the same row having different superscripts differ significantly (P < 0.05).
123 Values in the same column having different superscripts differ significantly (P < 0.05).
The values indicated were the mean of three trials ± SE (Standard Error).

The sensitivity of aerobic spore formers including Gram-positive and Gram-negative bacteria towards different bacteriocins has been demonstrated on the basis of cell wall composition. The relatively

resistance of Gram-negative bacteria is attributed to the particular nature of their cellular envelope with composition of lipopolysaccharide, protein and phospholipids and the size of the pores membrane. However, bacteriocin changes the permeability barrier proprieties of the outer membrane thereby dissipating ion gradients and inhibiting transport of amino acids to cells (**Abee et al., 1995 and Prada et al., 2007**).

The addition of bacteriocins during food production with suitable concentration has the ability for inhibiting the growth of undesirable spoilage and many serious food pathogens; such as bacillus, pseudomonas, listeria and clostridia species that make them ideal candidates to improve food safety and quality (**Galvez et al., 2007**).

3.4. Effect of bacteriocin on psychrotrophic bacteria

Owing to refrigerated storage of the pasteurized milk, some psychrotrophs can grow well at 7℃ or less. They deserve special attention, because they include many pathogens that cause infections, produce toxins as well as spoilage (**ICMSF, 1980**).

The main psychrotrophic genera isolated from milk are pseudomonas, bacillus, micrococus and lactobacillus (**Cousin, 1982**). The contamination of milk with psychrotrophs is either from the environment of dairy farm such as water, soil, utensils and faecal matter (**Eneroth et al., 1998**) or commonly enters the processed dairy products through post-pasteurization contamination (**Ralyea et al., 1998**). A typical psychrotrophic bacteria is pseudomonas strain that can produce heat-stable extracellular lipases, proteases and lecithinases leads to bitter and rancid flavour with clotting and soapy appearance (**Sorhaug & Stepanik, 1997 and Dogan & Boor, 2003).** *Bacillus cereus* species is one of psychrotrophs that consider as one of the most important organisms limiting the shelf-life of pasteurized milk stored above 6℃ and reduce its quality (**Griffiths, 1992 and Marth and Steele, 2001**).

Bacteriocin treated pasteurized milk samples of T1 showed the best role in controlling the psychrotrophic count extended to be not detected at 12th day of storage. While, those treated samples by low concentrations of either T3 or T4 as well as control samples of T5 showed either slight or no effect on psychrotrophic counts with the mean counts (log_{10} CFU /mL) of 4.16 ± 0.17 at the 9th day, 3.96 ± 0.17 and 5.83 ± 0.08 at the 6th day of refrigerated storage; respectively (**Table 4**).

The bactericidal effect of the bacteriocin produced by *L. acidophilus* on psychrotrophs was accompanied by bacterial lysis due to its ability to make changes in the structure of the cell wall leads to cell rupture, escaping of the cell contents and the cell wall finally disintegrated (**Ennahar et al., 1996 and Eijsink et al., 1998).** On the other hand, **Huang (1999)** reported that bacteriocins are highly hydrophobic and have ability to interact with anionic lipids of bacterial cell membrane.

Applying of good manufacturing practices (GMP) should be applied at both farm and plant level to monitor the initial microbial count of raw milk.

During the first half of the 20th century, the best known bacteriocins is nisin produced by *Lactococcus lactis*, is one of the most intensively studied lantibiotics, which has been generally recognized as safe (GRAS) that proved by WHO in 1969 and FDA in 1988 owing to its industrial and medical applications (**Delves-broughton et al., 1996, Chen & Hoover, 2003 and Deegan et al., 2006).**

Table (4): Effect of different concentrations of bacteriocin on psychrotrophic bacteria (Log_{10} CFU/ ml) in pasteurized milk samples

Storage time	T1	T2	T3	T4	T5
Zero time	2.00 ± 0.08^{A2}	2.11 ± 0.03^{A1}	2.30 ± 0.17^{B1}	2.10 ± 0.05^{AB1}	2.13 ± 0.17^{AB1}
3rd day	2.66 ± 0.06^{A3}	2.80 ± 0.05^{B3}	2.50 ± 0.10^{A1}	3.73 ± 0.24^{C2}	3.63 ± 0.14^{C2}
6th day	1.86 ± 0.21^{A2}	2.00 ± 0.15^{B2}	3.50 ± 0.17^{C2}	3.96 ± 0.17^{C2}	5.83 ± 0.08^{D3}
9th day	1.01 ± 0.05^{A1}	1.36 ± 0.14^{A1}	4.16 ± 0.17^{B3}	S	S
12th day	ND	1.95 ± 0.14^{B2}	S	S	S
15th day	ND	S	S	S	S

T1: Pasteurized milk with bacteriocin at concentration 320 IU/ ml
T2: Pasteurized milk with bacteriocin at concentration 160 IU /ml
T3: Pasteurized milk with bacteriocin at concentration 80 IU/ ml
T4: Pasteurized milk with bacteriocin at concentration 40 IU/ ml
T5: Pasteurized milk without bacteriocin (control)
ND: not detected
S: Samples showed spoilage
ABC Values in the same row having different superscripts differ significantly ($P < 0.05$).
123 Values in the same column having different superscripts differ significantly ($P < 0.05$).
The values indicated were the mean of three trials ± SE (standard Error).

4. Conclusion

The present study demonstrated that the bacteriocin extracted from *L. acidophilus* should have much attention as it could be another dietary adjunct to be approved by the international communities to be safely applied as its commercial application is still limited. As it plays an important role in controlling the total bacterial count, aerobic-spore formers and psychrotrophic bacterial counts in pasteurized milk. Moreover, it can extend shelf-life of pasteurized milk up to 12 days especially at concentration 320 and 160 IU/mL. While, low temperature short time pasteurization did not significantly affect the bacteriological profile of milk.

Corresponding author
Ekbal M. A. Ibrahim
Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt
ekbal_adel_2200 @yahoo.com

References

APHA "American Public Health Association" (1992): Standard methods for the examination of dairy products. INC, 16 th ed., New York.

Abee, T.; Krockle, L.; and Hill, C. (1995): Bacteriocins: Mode of action and potentials in food preservation and control of food poisoning. Int. J. of Food Microbiol., 28: 169-185.

Branen, J. K. and Davidson, P. M. (2004): Enhancement of nisin, lysozyme and monolaurin antimicrobial activities by ethylene diaminetetraacetic acid and lactoferrin. Int. J. of Food Microbiol., 90: 63–74.

Bizani, D.; Morrissy, J.A.; Dominguez, A.P. and Brandelli, A.(2008): Inhibition of *Listeria monocytogenes* in dairy products using the bacteriocin-like peptide cerein 8A. Int. J. of Food Microbiol., 31: 229-233

BogovicMatijasic, B.; Rogelj, I.; Nes, I. F. and Holo, H. (1998): Isolation and characterization of two bacteriocins of *Lactobacillus acidophilus* LF 221. Appl. Microbiol. and Biotechnol., 49: 606-612.

CFDRA" Campden Food and Drink Research Association"(1992): Food pasteurization. *Technical Manual* No-27, UK.

Chen, H. and Hoover, D. G. (2003): Bacteriocins and their food applications. Comprehensive Reviews . Food Sci. and Food Saf., 2: 82-100.

Chumchalova, J.; Stiles, J.; Josephsen, J. and Plockova, M. (2004): Characterization and purification of acidocin CH5, a bacteriocin produced by *Lactobacillus acidophilus* CH5. J. of Appl. Microbiol., 96: 1082-1089.

Clarke, G. M. and Kempson, R. E. (1997): Introduction to the design and analysis of experiments. Arnold, a Member of the Holder Headline Group. 1 st ed., London, UK.

Cleveland, J.; Montville, J. T.; Nes, I. F. and Chikinidas, M. L. (2001): Bacteriocin safe natural antimicrobials for food preservation. Int. J. of Food Microbiol., 17:1-20.

Cousin, M.A. (1982): Presence and activity of psychrotrophic microorganisms in milk and dairy products: A review. J. of Food Prot.,45: 172-207.

Deegan, L. H.; Cotter, P. D.; Hill, C. and Ross, P. (2006): Bacteriocins: Biological tools for bio-preservation and shelf-life extension. Int. Dairy J., 16: 1058–1071.

Delves- Broughton, J. P.; Blackburn, R. J. and Evans, Hugenholtz, J. (1996): Applications of the bacteriocin nisin. Antonie Van Leeuwenhoek 69: 193-207.

Dogan, B. and Boor, K.J. (2003): Genetic diversity and spoilage potentials among pseudomonas spp. isolated from fluid milk products and dairy processing plants. J. of Appl. and Environ. Microbiol., 96: 130-138.

Eijsink, V. F.; Skeie, M.; Middelhoven, P.H.; Bruberg, M. B. and Nes, I.F. (1998): Comparative studies of class II a bacteriocins of lactic acid bacteria. J. of Appl. and Environ. Microbiol., 64: 3275-3281.

Eneroth, A.; Christiansson, A.; Brendehaug, J. and Molin, G. (1998): Critical contamination sites in the production line of pasteurized milk with reference to the psychrotrophic spoilage flora. Int. Dairy J., 9**:** 929-834.

Ennahar, S.; Aoude-Werner, D.; Sorokine, O.; Van Dorsselaer, A.; Bringel, F.; Hubert, J. C. and Hasselmann, C. (1996): Production of pediocin AcH by *Lactobacillus plantarum* WHE 92 isolated from cheese. J of Appl. and Environ. Microbiol., 62: 4381-4387.

Ennahar, S.; Sashihara, T.; Sonomoto, K. and Ishizaki, A. (2000): Class IIa bacteriocins; biosynthesis, structure and activity. FEMS Microbiol. Rev., 24: 85-106.

EOSQ" Egyptian Organization for Standardization and Quality" (2005): Pasteurized milk. Egyptian Organization for Standardization and Quality. No. 1623.

Galvez, A.; Abriouel, H.; Lopez, R. and Ben Omar, N. (2007): Bacteriocin-based strategies for food biopreservation. Int. J. of Food Microbiol., 120: 51-70.

Griffiths, M. W. (1992): *Bacillus cereus* in liquid milk and other milk products. International Dairy Federation Bulletin. Int. Dairy Federation, Brussels, Belgium, pp.36-39.

Hassan, N. B.; Abdalla, M. O. and Nour, A. A. (2009): Microbiological quality of heat-treated milk during storage. Pakistan J. of Nutr., 8: 1845-1848.

Huang, H. W. (1999): Peptide-lipid interactions and mechanisms of antimicrobial peptides. Novartis Foundation Symposium, 225: 188-200.

ICMSF "International Commission on Microbiological Specifications for Foods " (1980): Factors affecting Life and Death of Microorganisms. In: Microbial Ecology of Foods. Vol.I. Academic Press, Toronto.

IDF "International Dairy Federation" (1988): Fermented milks: Science and Technology. Bulletin of the IDF: In A. No-227, Brussels.

Korhonen, H. Pihtanto- Leppala; Rantamaki, P. and Tupasella, T. (1998): Impact of processing on bioactive proteins and peptides. Trends Food Sci. and Technol., 9: 307-319.

Marth, E. H. and Steele, J. L. (2001): Applied Dairy Microbiology. 2 nd ed. Revised and Expanded. Marcel Decker, INC, New York, pp. 65.

Mc Auliffe, O.; Ross, R. P. and Hill, C. (2001): Lantibiotics structure, biosynthesis and mode of action. FEMS Microbiol. Rev., 25: 285-308.

Muñoz, A.; Ananou, A.; Gàlez, A.; Martinez- Bueno, M.; Rodriguez, A.; Maqueda, M. and Valdivia, E. (2007): Inhibition of *Staphylococcus aureus* in dairy products by enterocin AS-48 produced in situ and ex situ: bactericidal synergism through heat and AS-48. Int. Dairy J., 7: 760-769.

Nattress, F. M.; Yost, C. K. and Baker, L. P. (2001): Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. Int. J. of Food Microbiol., 22: 111–119.

Oh, S J.; Heo, H. J.; Park, D. J.; Kim, S. H. and Lee, S. J. (2006): Effect of encapsulated bacteriocin on acid production and growth of starter cultures in yoghurt. J. of Food Sci. and Biotechnol., 15: 902-907.

O'Sullivan, L.; Ross, R. P. and Hill, C. (2002): Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. Biochimie, 84: 593–604.

Pearson, D. (1984): Chemical analysis of foods. 8 th ed., Churchill Livingstone, Edinburgh, London.

Prada, J. L.; Caron, C. R.; Medeiros, A. B. and Soccol, C. R.(2007): Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. Brazilian Archives of Biol. and Technol., 50: 521-542.

Ralyea, R.D., Wiedmann, M., and Boor, K.J. (1998). Practical tracking in a dairy production system using phenotypic and ribotypic methods. J. of Food Prot., 61: 1336-1340.

Reij, M. W.; Den Aantrekker, E. D. and ILSI Europe Risk Analysis in Microbiology Task Force (2004): Recontamination as a source of pathogens in processed foods. Int. J. of Food Microbiol., 91: 1–11.

Rossi, L. M.; Rangasamy, P.; Zhang, J.; Qiu, X.Q. and Wu, G. Y.(2008): Research advances in the development of peptide antibiotics. J. of Pharm. Sci., 97: 1060-1070.

Røssland, E.; Andersen Borge, G. I.; Langsrud, T., and Sørhaug, T. (2003): Inhibition of *Bacillus cereus* by strains of Lactobacillus and Lactococcus in milk. Int. J. of Food Microbiol., 89: 205-212.

Ruegg, P. L. and Reinemann, D. J. (2002): Milk quality and mastitis tests. University of Wisconsin Extension Services. The Bovine Practices, 36:1-13.

Saavedra, L.; Minahk, C.; Holgado, A. P. and Sesma, F. (2004): Enhancement of the enterocin CRL35 activity by a synthetic peptide derived from the NH2-terminal sequence. Antimicrobial Agents and Chemotherapy, 48: 2778-2781.

Sable, S.; Pons, A.; Gendron-gaillard, S. and Cottenceau, G. (2000): Antibacterial activity evaluation of Microcin J25 against diarrheagenic *Escherichia coli*. .J. of Appl. and Environ. Microbiol., 66: 4595-4597.

Scheldeman, P.; Herman, L.; Goris, J.; Vos, P. and Heyndrickx, M. (2002): Polymerase chain reaction identification of *Bacillus sporotherodurans* from dairy sources. J. of Appl. Microbiol., 95: 983-991.

Scheldeman, P.; Pil, A.; Herman, L.; Vos, P. de. and Heyndrickx, M. (2005): Incidence and diversity of potentially highly heat resistant spores isolated at dairy farms. J. of Appl. and Environ. Microbiol., 71(3) :1480-1494.

Shai, Y.(2002): Mode of action of membrane active antimicrobial peptides. *Biopolymers* 66**:** 236-248.

Simova, E. D.; Beshkova, D. B. and Dimitorv, Z. P. (2009): Characterization and antimicrobial spectrum of bacteriocins produced by lactic acid bacteria isolated from traditional Bulgarian dairy products. J. of Appl. Microbiol., 106: 692-701.

Sorhaug, T. and Stepanik, L. (1997): Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. Trends in Food Sci. and Technol., 8: 35-40.

Taher, Z. M. A. (2004): Studies on Bacillus cereus and related species in heat-treated milk and some milk products. MVSc. Thesis, Assuit Univ., Fac. of Vet. Medicine.

Todorov, S. D. and Dicks, L. M. T. (2004): Comparison of two methods for purification of plantaricin ST31, a bacteriocin produced by *Lactobacillus plantarum* ST31. Enzyme and Microbiol. Technol., 36: 318-326.

U.S. Public Health Service (1995): Grade A Pasteurized Milk Ordinance. Publication No.229, Food and Drug Administration, Washington DC.

Viedma, P. M.; Abriouel, H.; Omar, N. B.; López, R. L.; Valdivia, E. and Gálvez, A. (2009): Assay of enterocin AS-48 for inhibition of foodborne pathogens in desserts. J. of Food Prot., 27: 1654-1659.

Vyletelova, M.; Hanovs, O.; Urbanova, E. and Kopunecz, P. (2000): The occurance and identification of psychrotrophic bacteria with proteolytic and lipolytic activity in bulk milk samples at storage in primary production conditions. J. of Anm. Sci., 45: 373-383.

Vyletelova, M.; Svec, P.; Pacova, Z.; Sedlacek, I. and Roubal, P. (2002): Occurrence of *Bacillus cereus* and *Bacillus licheniformis* strains in the course of UHT milk production. Czech J. of Anm. Sci., 47: 200-205.

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