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Utilization of Lactic Acid Bacteria Bacteriocin for Prolonging Shelf Life of Minced Meat

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Abstract: Raw meat is known to be a source of multiple microbial foodborne. Bacteriocins have received a lot of publicity as a natural and safe approach for the preservation of food. In this study, bacteriocin from lactic acid bacteria was evaluated as an effective preservative agent in extending the shelf life of minced meat. The bacteriocin was produced and extracted by pH mediated cell adsorption method from two strains of *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus.* The antibacterial activity was investigated by well diffusion and microdilution methods. The results showed that the antibacterial spectrum of both *L. bulgaricus* and *L. rhamnosus* products was broad and effective not only against Gram-positive such as *B. cereu*s but also against Gram-negative bacteria such as *E. coli* and *S. typhimurium*. Minimum inhibition concentrations (MIC) ranges for *L. bulgaricus*-partially purified bacteriocin (BLB) were between 1.25 and 0.625 mg/ml, while for *L. rhamnosus* (BLR) were between 2.5 to 0.078 mg/ml. Almost all tested pathogenic bacteria could be inhibited at a dilution of 1:4 (1.25 mg/ml) of bacteriocin of either *L. bulgaricus* and *L. rhamonosis*, so this concentration was used for preservation of fresh minced meat. From microbiological examination it was noticed that the control sample became unacceptable after 6 days of storage at 4°C, total count bacteria recorded 8.2x 10^7 CFU/g, whereas A (contains BLR) and B (contains BLB) samples were acceptable until 8 days of storage, with lower total count bacteria of (1.0x 10^4 and 9.6x 10^4 CFU/g) and (2.3 x 10^5 and 7.1 x 10^5 CFU/g) after 6 and 8 days of storage, respectively. The obtained results showed that control samples (C) reached to spoilage level of psychrophilic bacteria after 6 days of storage, while the treated meat samples with bacteriocin recorded (7.7 x 10^2 and 3.4 x 10^3) and (2.3 x 10^4 and 2.6 x 10^5) CFU/g after 6 and 8 days of storage at 4°C for A and B samples. Also, the control sample was spoiled (as chemical quality attributes) after storage for 6 days at 4°C, where recorded 28.25 mg/100g for TVN. On other hand, A and B samples were below the permitted level, where recorded 17.90 and 19.20 mg/100g after stored for 8 days at 4°C, respectively. It was concluded that bacteriocin can be effectively used to extend the shelf life of minced meat.

Key words: Bacteriocin · Minced Meat · Meat Preservation · A bio preservative agent · Lactic acid bacteria · Shelf Life · Minced Meat

INTRODUCTION

Fresh minced meat I s highly preferred by consumers due to its perishable characteristics, but the handling process and exposure to the air surrounding temperature make it extremely vulnerable to bacterial contamination. The incidents of spoilage species in meat cause undesirable odors, bad flavors, changes in color and production of slime and gas. To date, industrial chemical preservatives are used excessively to reduce the growth of microorganisms in foods [1]. These chemicals include sulfites, sulfur dioxide, nitrates, nitrites, Sodium diacetate, β-propiolactone, benzoic acid, ascorbic acid and antibiotics, which are progressively being doubted about their side effect on human health [2].

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There is always stress on the food handling industry every day for the discovery of safe preservatives that satisfy consumer demands, this led to a trend towards using green technology for employing natural alternatives in food preservation to boost the shelf-life of red meat [3]. In this regard, increased attention was forwarded to bacteriocins. Bacteriocins are antibacterial peptides biosynthesized mostly by the lactic acid bacteria group (LAB) [4]. They are widely recognized as safe and non-cytotoxic substances to eukaryotic cells. They have bactericidal and/or bacteriostatic activity, with little influence on the intestinal flora. Bacteriocins can also be a promising solution to the increasing problem of microbial resistance to antibiotics [5], since bacteriocins killing mechanism is dependent on disturbing membrane integrity and their fragment does not interfere with the cell of the target organism, so they are less possible to induce resistance [6]. This makes it ideal for food preservation [7].

Bacteriocins are often employed as fermentation products of the producer strain or as an extracted from either in a partially-purified or purified preparation [8]. The only commercially available bacteriocins to date are nisin and pediocin PA-[9, 10]. In terms of its utility in the preservation of meat, nisin has certain drawbacks like low solubility, the risk of enzymatic destruction, as well the incompetency of antibacterial efficacy towards certain bacterial species [9]. That made pediocin PA-1 came primarily in the meat applications [7]. This bacteriocin can decrease the proliferation of spoilage microbial population throughout storage [11]. Pentocin 31-1 and the partially-purified bacteriocins BacTN635 and BacFL31have also revealed their usefulness in the protection of various meat products [12-14].

Lactobacillus rhamnosus is an optional heterofermentative lactic acid bacterium (LAB) [15]. While the famous strain in the yogurt industry-*Lactobacillus delbrueckii subsp. Bulgaricus* is a homofermentative strain [16]. These species are generally recognized as safe (GRAS) and are well known for their probiotic properties [17, 18]. Although some researchers documented the antibacterial ability of *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* [19-21]. There have been few reports on the application of these organisms in the field of meat preservation. Hence, the aim of this study was to exploit the extracted bacteriocin produced from two strains of LAB namely *Lactobacillus rhamnosus* and *Lactobacillus bulgaricus* and study its effectiveness as a bio preservative agent in extending the shelf life of minced meat.

MATERIALS AND METHODS

Bacterial Strains and Culture Preparations: In this study, six food-borne pathogens bacteria and two lactic acid bacteria strains were used. Food-borne pathogens bacteria include two-gram positive bacteria: *Staphylococcus aureus* DSM 20231 and *Bacillus cereus* ATCC 33018 and four Gram-negative bacteria: *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* ATCC 13315, *Escherichia coli* ATCC 69337, *Shigella* spp and *Listeria* spp. Meanwhile, lactic acid strains including *L. rhamnosus* NRRL B-1445 and *L. delbrueckii* subsp. *bulgaricus* NRRL B-545, originated from the ARS Culture Collection (NRRL). These strains were obtained from the National Research Center, Giza, Egypt. *Lactobacillus* strains were grown and maintained on Man Rogosa Sharpe (MRS) (Biolife) and were incubated anaerobically (Oxoid Gas Generating Kit) at 37°C for 24hrs. While the pathogenic strains were grown and maintained on nutrient agar slants (TSA, Merck) and incubated aerobically at 37°C for 24hrs.

Antibacterial Activity Determination by Well Diffusion Assay: Antibacterial activity of LAB strains against foodborne pathogenic bacteria was determined by the well diffusion method using Mueller- Hinton agar (MHA). For the preparation of food borne pathogenic bacteria, bacterial culture was incubated at 37°C for 24 hrs. After incubation, the indicator bacteria strains were suspended in 5ml of sterile saline and then adjusted by comparing against 0.4-0.5 McFarland scale standard. These suspensions (100 µl) (1.5×10^8 cfu/ml) were then diluted in 0.9% saline to give 10^6 cfu/ ml and 100 µl aliquots of each prepared bacterial suspension were spread on the agar plates. For preparation of cell free-extract (CFE) and crude bacteriocin, *Lactobacillus* (18hr) cultures were centrifuged (4.470xg at 4°C for 15 min) and the CFE (fraction 1) was adjusted to pH 6.0-6.5 by the addition of 1.0 N NaOH, heated to 70°C for 25 min to inactivate proteases and filtered (0.45 µm pore diameter filter, Millipore, Billerica MA, USA) to obtain crude extract. 50 µl of each of CFS and crude extract was added into each well. All plates were incubated for 16-18 h at 37° C (TE-310, Tecnal, Piracicaba, SP, Brazil) in duplicate. The diameter of the inhibition zone (mm) was measured after overnight incubation [22].

Purification of Bacteriocin: Adsorption of bacteriocin to the producer cells was performed using the pH-mediated cell adsorption-desorption method described by

Yang *et al*. [23] and Mbawala *et al*. [24] with minor modifications. The culture broth was heated for 30 min at 70°C to prevent the inactivation of the bacteriocin by proteases. Next, the culture was adjusted to pH 6.0 with 1 M NaOH and stirred for 30 min at room temperature to allow absorption of the bacteriocin to the producer cells. The cells were then collected by centrifugation (16, 000 rpm, 15 min, 4°C) and washed twice with sterile 0.1 M phosphate buffer (pH 6.5). The pellets were suspended in 100 mM NaCl, adjusted to pH 2.0 with 1 M HCl and then stirred for 12 hrs at 4°C. The cell suspensions were then centrifuged at 16, 000 rpm for 25 min and the supernatants were filter-sterilized (fraction 3) and then lyophilized. Protein concentration was determined by spectrophotometer. The concentration of the protein is determined spectrophotometrically according to Layne [25] methods and calculated according to the following formula:

Concentration (mg/ml) = (1.55 x A280) – (0.76 x A260)

where A280 and A260 are the absorbance of the sample at a wavelength of 280 nm and 260 nm, respectively.

Determination of Minimum Inhibitory Concentration and Antibacterial Activity of Bacteriocin: Minimum inhibitory concentrations (MIC) of extracts against the tested pathological bacterial strains were determined using the broth microdilution method as described by Qaiyami *et al*. [26] and Elshikh *et al*. [27]. Briefly, serial two-fold dilutions of bacteriocin- fraction 3 solution (10-0.039% mg/ml) were prepared in 96-well micro-titer plate containing 50 µl of Mueller-Hinton broth (Merck, Darmstadt, Germany) from column 1 to 9. Column 12 and 11 contained 50 µl of diluted two-fold broth media and Column 10 contained 50 µl of the medium broth (as a control to monitor sterility), as shown in processed plate Fig. 2. The standardized indicator strains suspension was then diluted by 1:100 in MHB broth to give 10^6 cfu/ml. 10 µl of the adjusted OD_{600} bacterial suspension was then added to all wells containing bacteriocin and to the control wells in columns 11 and 12, resulting in approx. 5×10^5 CFU/ml. After incubation for 24 hr at 37°C, resazurin (0.015 %) was added to all wells (30 µl per well) and further incubated for 2-4 h for the observation of the color change. On completion of the incubation, wells with no color change (blue resazurin color remained unchanged) were scored as the MIC value. The activity was expressed as an Arbitrary Unit (AU) per ml calculated from the reciprocal of the highest dilution of the bacteriocin preparations which give blue color (indicating inhibition of growth). Arbitrary Unit ml^{-1} was calculated according to following formula: (1000/50) $*2^b$, whereas b is the number of wells giving blue color for each indicator strain.

The Effect of the Bacteriocin on the Shelf Life of Minced Meat: Fresh meat was obtained from a local supermarket; all visible fat had been removed. Then meat was cut into smaller portions to ensure homogeneity and minced. The minced meat was divided into three groups, the first group (A) was treated with 1.0% concentration of the semi-purified bacteriocin from *L. rhamnosus*, the second group (B) treated with 1.0% concentration of the bacteriocin from *L. bulgaricus*, the third group control (C) without bacteriocin treatment. The samples of meat were stored for 12 days at 4°C. The samples were analyzed each (0, 3, 6, 8, 10 and 12) days.

Chemical Quality Attributes Evaluation: Total volatile nitrogen (T.V.N) and pH value were determined inconsistent with AOAC [28]. All results were expressed as a mean of triplicates.

Microbiology Evaluation: For microbiological assessment, 5 grams of minced meat were taken from each treated sample, homogenized and diluted with buffered peptone water to make dismal dilutions [29]. 1 ml of each concentration was plated in petri plates and the appropriate media was poured over it and carefully mixed. Bacterial count Agar (Biolife, USA) was used to estimate both total count bacteria (TBC) and psychrophilic bacteria [30]. Accordingly, plates were incubated at 30°C for 24-72 hr for TBC or at 7°C for 10 days for psychrophilic bacteria. For yeast and mold counts (YM), supplemented Rose-Bengal Chloramphenicol agar (Oxoid, USA) was used [31] followed by incubation at 25°C for 3-5 days. The cfu g^{-1} of the samples was counted at various time intervals (0, 3, 6, 8, 10 and 12 days). All results were expressed as a mean of three replicates.

Statistical Analysis: The obtained data were exposed to the analysis of variance followed by multiple comparisons between means ($P \leq 0.05$) applying LSD. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System SAS [32].

RESULTS AND DISCUSSION

In order to determine the spectrum of antimicrobial activity of the antibacterial metabolites produced by

L. bulgaricus and *L. rhamnosus*, the cell-free supernatant (fraction 1) and crude bacteriocin (fraction 2) were tested against Gram-positive and Gram-negative food borne pathogenic bacteria by well diffusion method (Table 1). The antimicrobial spectrum of the LAB was broad and effective not only against Gram-positive like *B. cereus* but also against Gram-negative bacteria such as *E. coli* and *S. typhimurium.*

On the other hand, it could be noticed from Fig. (1) that *L. bulgaricus*' supernatants showed higher inhibition zone than that belonging to *L. rhamnosus*. The Highest inhibition zone is recorded by *L. bulgaricus* supernatant against *Staph. aureus* (30 mm). While crude bacteriocin of *L. rhamnosus* recorded a higher inhibition zone than *L. bulgaricus*. Furthermore, *B. cereus* and *Staph. aureus* were the most sensitive strains to crude bacteriocin of *L. rhamnosus* with an inhibition zone of 18 mm. The antimicrobial effect exerted by LAB might be caused by production of lactic acid, reduction of pH, diacetyl and hydrogen peroxide and other primary and secondary antimicrobial metabolites such as bacteriocin [33].

When comparing the supernatant and crude bacteriocin, it has been noted that the inhibition zone decreased against indicator bacteria, particularly with *L. bulgaricus*, which suggests that some of antimicrobial activity was due to the production of organic acid secretion [24]. The influence of antimicrobial activity given by organic acids including lactic acid, acetic and propionic, results from the action of the acids on the bacterial cytoplasmic membrane that interferes with the maintenance of membrane potential and hinders active transport [34].

Fig. 1: Antibiogram of lactic acid bacteria against *B. cereus*. 1, 4: supernatant and crude bacteriocin of *L. bulgaricus* subsp. *bulgaricus* NRRL B-548, respectively; 2, 3: supernatant and crude bacteriocin of *L. rhamnosus* NRRL B-1445, respectively; -ve: negative control, +ve: positive control; p: protease treated crude extract.

Table 1: Antibacterial activity (mm) of LAB strains against food borne pathogenic strains by well diffusion method

	LAB strains			
	L. bulgaricus		*L. rhamnosus*	
Pathogenic bacteria strains	S	C	S	C
B. cereus ATCC 33018	25	13	20	18
S. aureus DSM 20231	30	12	20	18
P. vulgaris ATCC 13315	25	12	23	14
E. coli ATCC 69337	20	10	16	10
S. typhimurium ATCC 14028	20	14	19	10
Shigella spp	25	8	18	8
Listeria spp	25	8	16	9

S: supernatant, C: crude bacteriocin

The supernatant of both LAB strains displays a wide range of inhibition against indicator strains, these results are also in accordance with previous work carried out by Mohamed *et al.* [35] who revealed that cell-free supernatant of lactic acid strains had a varying degrees of inhibition towards both Gram-positive and Gram-negative bacteria. Likewise, IErdourul and Erbulur [36] and Tufail, *et al.* [37] tested culture supernatants of *L. bulgaricus* and found that it exhibited inhibitory activity against strains of *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Vibrio cholera*.

Stevens *et al*. [38] hypothesized that the bacteriocins of LAB can poorly inhibit gram negative bacteria, because the outer membrane of these bacteria obstructs the site used by this bacteriocin. But in the current results, a crude bacteriocin from both strains was not only active against gram-positive but also against gram-negative bacteria. Bacteriocins from both two LAB strains were concentrated from the growth medium by a method based on the influence of pH on adsorption and release of the bacteriocin. After extraction of bacteriocin, it was found that it retained considerable antibacterial activity also. The protein concentration was higher in *L. rhamnosus* than *L. bulgaricus* which results in higher inhibition zones for *L. rhamnosus*.

In the current study, we exploit the microdilution method for determining the total antibacterial activity, it was revealed from Table (2) that almost all tested pathogenic bacteria could be inhibited at a dilution of 1:16 (320 AU/ml) of bacteriocin of either *L. bulgaricus* or *L. rhamnosus*. *B. cereus* and *Staph. aureus* were the most sensitive strains to bacteriocin of *L. rhamnosus*, as growth was completely inhibited after dilutions (5, 120 AU/mL), respectively, while the most sensitive strains to the bacteriocin of *L. bulgaricus* were *B. cereus* ATCC 33018 and *S. typhimurium* ATCC 14028 which was inhibited at dilution of 1: 5 (640 AU/ml).

Table 2: Comparison of MICs in mg/ml and antibacterial activity unit (Au/ml) of *lactobacillus* strains recorded against some food-borne pathogenic bacteria

	LAB strains					
	L. bulgaricus			*L. rhamnosus*		
Pathogenic bacteria Strains	MIC (mg/ml)	Activity/ AU/ml	Specific activity/ AU/mg Protein	MIC (mg/ml)	Activity/ AU/ml	Specific activity/ AU/mg Protein
B. cereus ATCC 33018	0.625	640	719	0.078	5120	1917
Staph. aureus DSM 20231	1.25	320	359.5	0.078	5120	1917
Pr. Vulgaris ATCC 13315	1.25	320	359.5	0.15	2650	992.5
*E. coli*ATCC 69337	1.25	320	359.5	1.25	320	119.8
S. typhimurium ATCC 14028	0.625	640	719	2.5	160	59.9
Shigella spp	1.25	320	359.5	1.25	320	119.8
Listeria spp	1.25	320	359.5	1.25	320	119.8

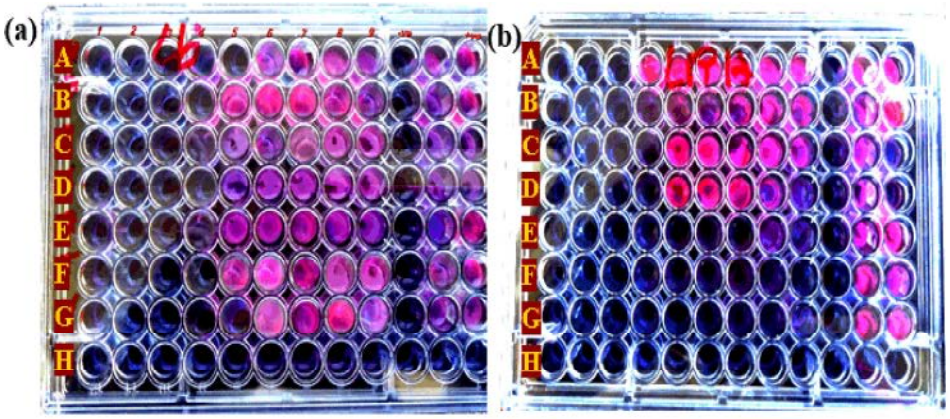

Fig. 2: Determination of MIC for bacteriocin by microdilution method. (a) *L. bulgaricus* subsp. *bulgaricus* NRRL B-548 (b) *L. rhamnosus* NRRL B-1445 against indicator strains (rows A-G): A: *S. Typhimurium* ATCC 14028, B: *E. coli* ATCC 69337, C: *Listeria* spp, D: *Shigella* spp, E: *Pr. vulgaris* ATCC 13315, F: *Staph. aureus* DSM 20231, G: *B. cereus* ATCC 33018. (columns 1-9): bacteriocin's concentrations

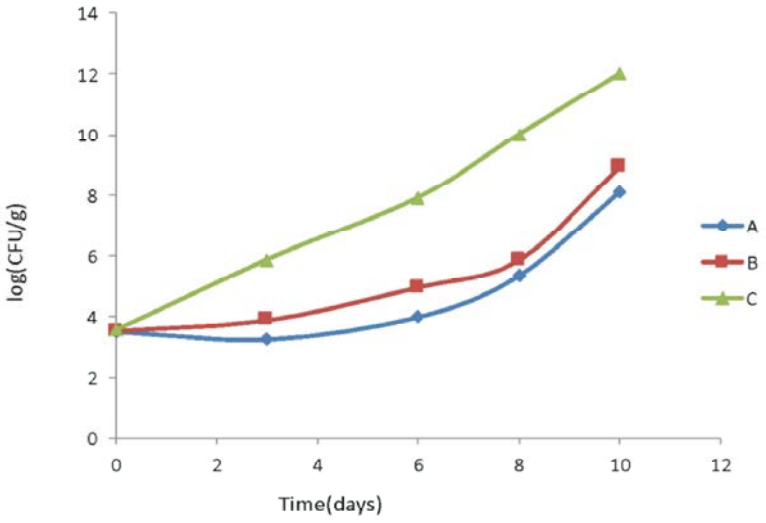

Fig. 3: Total bacterial count (Log CFU/g) of control (C) and treated samples (A and B) of minced meat stored at 4°C for 10 days
(A) = meat samples were treated with (1.0% w/v) of bacteriocin from *L. rhamnosus*,
(B) = meat samples were treated with (1.0% w/v) of the bacteriocin from *L. bulgaricus*,
(C) = without bacteriocin (control).

MIC is used to assess the antimicrobial capability of bacteriocins. For *L. bulgaricus* (BLB), the MIC range was between 1.25 and 0.625 mg/ml, while for *L. rhamnosus* (BLR) it was between 2.5 to 0.078 mg/ml. Generally, BLB showed more inhibitory activity than BLR. Range of this MIC has been reported in bacteriocin F1 by Miao *et al.* [39]; BacCH91 by Wladyka *et al*.[40]; nisin by Iancu *et al*. [41] and bacteriocin MN047A by Lanhua *et al*. [42].

In harmony with our results, Kim *et al*. [43] extracted the antimicrobial substance produced by *L. bulgaricus* and it was found active against both gram-positive and gram-negative pathogens with activity of 320 AU/ml recorded against *Staph. aureus* ATCC6538. The molecular mass of the bacteriocin was about 14 kDa. Srinivasan *etal*. [44] purified a bacteriocin from *L. rhamnosus* isolates and found that it exhibited inhibition against food-borne pathogens and spoilage microorganisms, including both Gram-positive and Gram -negative bacteria. In this context, Marie *et al*. [45] isolated *L. rhamnosus* 1K and found that it produces bacteriocin which is active against a wide range of gram positive and negative bacteria with a maximum bacteriocin activity of 3200 AU/ml.

In the current study, to evaluate the effectiveness of the incorporation of bacteriocin in minced meat for improving its shelf life, the total bacteria counts were determined in samples untreated and treated with bacteriocin that were stored 10 days under refrigeration (Figure 3). These values show that the initial microbiological quality of meat was acceptable at zero time of storage in all samples. But, the total count bacteria after the first 3 days of storage appeared to slightly increase in A and B samples were recorded $1.0x10^3$ and $8.1x\ 10^3$ CFU/g, whereas the control sample recorded $7.5x\ 10^5$ CFU/g. It could be noticed that after 6 days of storage, the control sample recorded $8.2x10^7$ CFU/g, it is clear that it became unacceptable according to Egyptian Organization Standardization [46]. Whereas, A and B samples recorded ($1.0x\ 10^4$ and $9.6x\ 10^4$ CFU/g) and ($2.3\ x\ 10^5$ and $7.1\ x\ 10^5$ CFU/g) after 6 and 8 days of storage, it is clear that treated samples (A, B) were acceptable and under the limit described in Egyptian Organization Standardization [46] which pointed that minced meat should not contain total count bacteria more than $1.0\ x\ 10^6$ CFU/g. These results are related to the inhibitory action by this antimicrobial extract' treated samples. Whereas after 10 days, the total bacteria count reached the unacceptable limit of all samples. These results are similar with results obtained by Maria and Rosalía [47], who reported that the total bacteria count in samples treated with the bacteriocin extract after 6 days of incubation was lower than in the control. Similarly, Gertruida [48] showed that the microbiological spoilage limit of untreated meat was reached after 6 days, while that of the bacteriocin-treated sample was reached after 8 days.

In meat packaging field, researchers have additionally ascertained an identical reduction within the total populations of mesophilic bacteria, when meat samples are packed with materials containing bacteriocins [49]. Also Guerra *et al.* [50] studied the influence of nisin adsorption to cellophane, reporting that the final level of total bacterial count after 12 days of incubation (approximately $7.9\ x\ 10^3$ CFU/g) was considerably lower than that of the total count of bacteria in the initial level, revealing that the bioactive substances provided good protection against bacterial growth. On the other hand, Ercolini *et al.* [51] found that total bacteria count was not reduced by the use of nisin adsorbed- package film in the first 5 days, but after 22 days and until the end of time storage, they remained 2 log units lower than control values.

From the obtained results in Figure (4), it could be noticed that control samples (C) reached to spoilage level of psychrophilic bacteria after 6 days of storage, while the treated meat samples with bacteriocin recorded ($7.7\ x\ 10^2$ and $3.4\ x\ 10^3$) and ($2.3\ x\ 10^4$ and $2.6\ x\ 10^5$) CFU/g after 6 and 8 days of storage for A and B samples, respectively. It is clear that psychrophilic bacteria level is not reached to the level allowance in treated meat samples (A, B) until 8 days of storage. Which indicates an inhibitory action by this antimicrobial extract during storage time in treated samples. These results conform to the obtained results by Maria and Rosalía [47] who showed that level spoilage of psychrophilic bacteria reached between 9 and 12 days of storage in meat treated with bacteriocin, while for the control these values were obtained between 3 and 6 days of storage. Fiorentini *et al.* [52] and Vázquez *et al*. [53] reported a reduction on psychrotrophic flora from treated meat with crude bacteriocin substances during 12 days of storage.

From Figure (5), it could be showed that the initial count of yeast and mold of minced meat was $4.5\ x\ 10^2$ CFU/g, which was down below the values reported by Maria and Rosalía [47] who indicated that counts of mold and yeast of $3.9\ x\ 10^3$ CFU/g, while Rai *et al.* [54] established initial count of yeast of 10^4 CFU/g. It could be noticed that with progress in the time of storage, the yeast and mold counts increase in all samples, but this increase in control samples (C) was higher when compared with B and A, respectively, at all period time of storage (3, 6, 8 and 10 days). This may be due to the inhibitory effect of bacteriocin in treated meat samples (B and A).

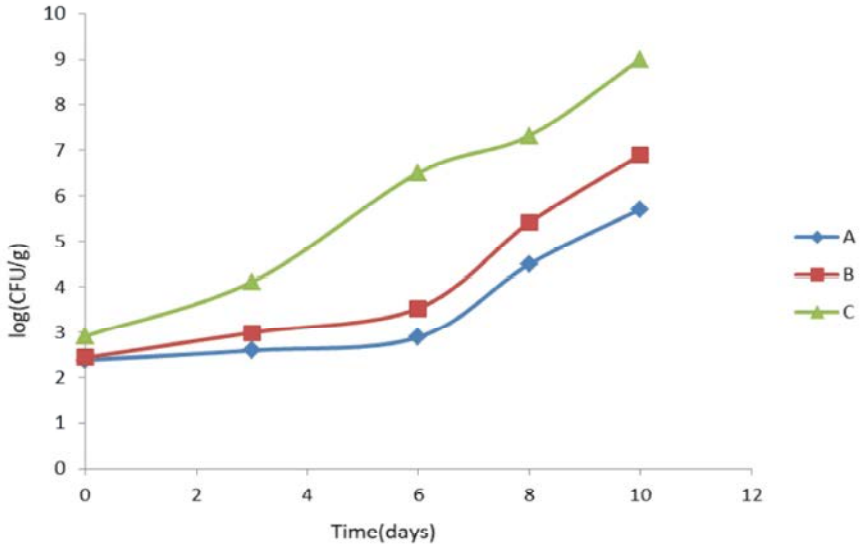

Fig. 4: Psychrophilic bacteria (Log CFU/g) of control (C) and treated samples (A and B) of minced meat stored at 4°C for 10 days
(A) = meat samples treated with (1.0% w/v) of bacteriocin from *L. rhamnosus*,
(B) = meat samples treated with (1.0% w/v) of the bacteriocin from *L. bulgaricus*,
(C) = without bacteriocin (control).

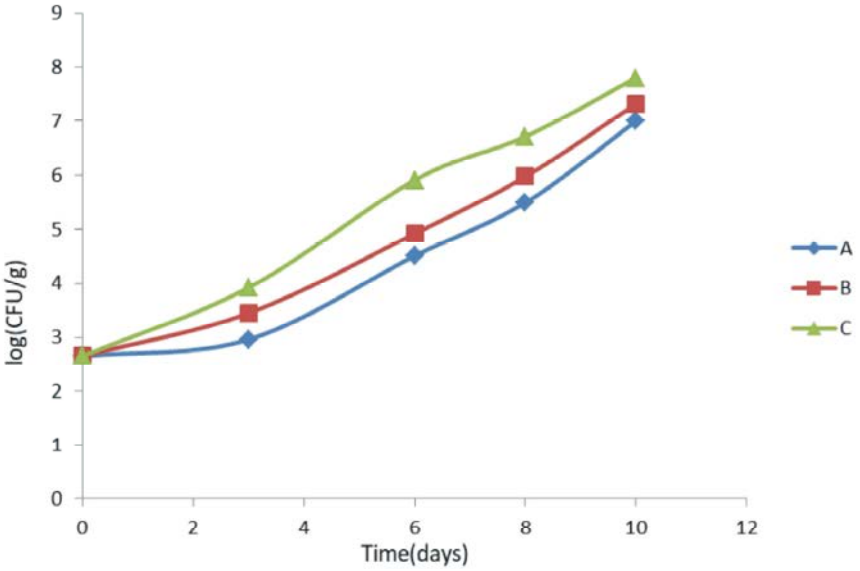

Fig. 5: Yeast and mold counts (Log CFU/g) of control (C) and treated samples (A and B) of minced meat stored at 4°C for 10 days
(A) = meat samples treated with (1.0% concentration) of bacteriocin from *L. rhamnosus*,
(B) = meat samples treated with (1.0% concentration) of the bacteriocin fom *L. bulgaricus*,
(C) = without bacteriocin (control)

Some authors suggested that the addition of the bacteriocin extract does not exert their antagonist action directly on these microbial populations, but their introduction into a complex food matrix may result in an imbalance in the natural flora and that inversely affect bacterial growth [55]. Other researchers report antimycotic properties of lactic acid bacteria strains and their bacteriocin. Bacteriocins were found to be effective in inhibiting both fungal growth and spore germination like BacTN635, nisin and *L. plantarum*' bacteriocins [56, 57].

Table 3: pH value of control and treated minced meat samples with bacteriocin during storage at 4°C for 10 days

	Days				
Samples	0	3	6	8	10
A	5.56^{a}±0.01	6.1^{c}±0.01	6.35^{c}±0.05	6.80^{c} ±0.05	8.10^{c}±0.03
B	5.52^{a}±0.02	6.3^{b}±0.01	6.65^{b}±0.04	6.95^{b} ±0.03	8.30^{b}±0.05
C	5.8^{a}±0.02	6.65^{a}±0.02	7.20^{a}±0.03	7.8^{a}±0.03	8.5^{a}±0.05
LSD	0.061	0.1780	0.1320	0.0819	0.1630

(A) = meat samples treated with (1.0% concentration) of bacteriocin from *L. rhamnosus*,

(B) = meat samples treated with (1.0% concentration) of the bacteriocin from *L. bulgaricus*,

(C) = without bacteriocin (control).

Table 4: TVN of control and treated minced meat samples with bacteriocin stored at 4°C for 10 days

	Days				
Samples	0	3	6	8	10
A	9.30^{a} ±1.40	10.35^{c}±1.20	13.60^{c}±1.00	17.90^{c}±1.35	33.55^{c}±1.25
B	9.30^{a} ±1.50	11.20^{b}±1.10	15.30^{b}±1.25	19.20^{b}±1.50	36.65^{b}±1.20
C	9.35^{a} ±1.50	18.65^{a}±1.15	28.25^{a}±1.30	35.20^{a}±1.55	40.75^{a}±1.20
LSD	0.163	0.716	1.348	0.807	1.260

TVN = Total volatile nitrogen (mg /100 gm).

(A) = meat samples treated with (1.0% concentration) of bacteriocin from *L. rhamnosus*,

(B) = meat samples treated with (1.0% concentration) of the bacteriocin from *L. bulgaricus*,

(C) = without bacteriocin (control)

pH measuring is considering the most important physicochemical parameter of meat quality. The pH of the meat may affect its color, tenderness and eating quality [58]. From the presented results in Table (3), it could be found that initial pH of control meat samples was 5.8 which decreased after adding bacteriocin extract to 5.56 and 5.52 in B and A samples, respectively. But with progress in time storage showed that pH of control meat samples was the highest when compared with treated meat samples (B and A) at any time of storage periods. These results were in harmony with Maria and Rosalía [47] and Fiorentini *et al.* [52] who found that, obtained initial pH of meat samples were 6.07 decreased to 5.81 after adding the cell-free bacteriocinogenic supernatants and remained at 6.08 for the control. In addition, Vázquez *et al.* [53] obtained an average initial value of pH 5.74, which after treatment with cell-free bacteriocinogenic supernatants remained at 5.69. After 6 days of storage, it showed an increase in the pH values and kept this value until 9 days of storage. Such increase in pH reflects the degree of deterioration of meat through the degradation of proteins with free amino acid production, leading to the formation of alkaline compounds as NH_3 and amines [59].

Results in Table (4) showed that T.V.N of both the control and treated minced meat samples with bacteriocin (A and B). It could be noticed that the control samples had the highest level of T.V.N followed by B and A samples, respectively. These results could be explained by increasing the total bacteria count and psychrophilic bacteria load of the samples C, B and A samples, respectively as shown in figure (3 and 4). On the other hand, from the same table (4) it could be found the amounts of T.V.N were gradually increasing with increasing of storage time at 4°C of all samples, but the control had the highest increasing rate followed by B and A samples, respectively. This may be due to the effect of antimicrobial activity of bacteriocin extract, which affected the bacteria activity and led to decrease in deterioration degree of protein alkaline compounds as NH3 and amines. According to Egyptian Organization Standardization [46], minced meat should not be contained T.V.N. more than 20 mg/100g (w/w). From data found in table (4), it could be noticed that control samples were spoiled after being stored 6 days at 4°C, where 28.25 mg/100g. On other hand, it is clear that this level did not exceed A and B samples and were below the permitted level and recorded 17.90 and 19.20 mg/100g after being stored for 8 days at 4°C, respectively. Also it could be noticed that B samples had higher values of T.V.N. than A. This may be due to bacteriocin activity potential of A was higher than B. While, the results showed that meat samples treated with the bacteriocin (A and B samples) reached to the level of spoilage between 8 and 10 days at 4°C.

CONCLUSION

Extensive research is currently being conducted to find new bacteriocins with a wider range of activities and food system compatibility. The antimicrobial compounds produced by *L. rhamnosus* NRRL B-1445 and *L. delbrueckii* subsp. *bulgaricus* NRRL B-548 strains have proved their efficacy as antibacterial candidates in cell free extract (CFE), crude, or semi-purified forms, thereby expanding their use. To the best of our knowledge, this is the research to investigate the application of the semi-purified bacteriocins extracted from the respective lactobacillus acid bacteria strains (LAB) in the meat preservation field and the findings highlight their effectiveness in extending the shelf life of the examined minced meat for 8 days compared with 6 days at 4°C for control samples. Based on microbiological and chemical characteristics. Therefore, this study proved the possibility of using the aforementioned strains as promising biopreservatives.

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