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Detection, Purification and Characterization of Newly Bacteriocin by Lactic Acid Bacteria Isolated From Dairy Products in Egypt, a Promising Approach in Food Biopreservation

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Abstract: Safe food selection of high nutritional value reflects on human health and life. Traditional processes implemented on preservation platforms to assure quality and safety food are usually not sufficient to eliminate pathogens and psychrotolerant microorganisms can survive during the prolonged shelf life of food. Biopreservation is an alternative technology used to extend shelf life and increase hygienic quality of food. Biological preservation relays on using of natural microflora and/or its antimicrobial metabolites to prolong shelf life and food safety. For this approach, Lactic acid producing bacteria (LAB) are promising candidates that produces wide range of antimicrobial metabolites as bacteriocins which indicated to prevent natural growth of undesirable bacteria in food. In this study, hundred samples of traditional fermented dairy products including Karish cheese (various cheese types), goat fermented milk and Laban Rayeb (concentrated sour milk) were collected from different places in Egypt. Isolation of lactobacilli was carried out using MRS and M17 media followed by biochemical identification. Totally fifty isolates were recovered from collected hundred dairy samples. Out of the 50 isolates, 30 were *Lactobacillus* spp., 10 were *Lactococcus* spp. and 10 were *Streptococcus* spp. Isolates were investigated for their probiotic properties as sodium chloride, lactic acid or hydrochloric acid and bile salt tolerant. Molecular characterization of *Lactobacillus* isolates was applied using primers PCR genus-specific designed to intergenic spacer region 16S/23S ribosomal RNA. Molecular size of bacteriocin from the culture supernatant of *L. plantarum* and *brevis* was determined using SDS-PAGE electrophoreses (10%) and subsequently the gel was stained with Coomassie blue. Protein band at molecular seizes of approximately 9, 12 kDa was obtained respectively.

Key words: 16SrRNA · Bacteriocin · Biochemical characterization · Dairy products and lactic acid bacteria

INTRODUCTION

Contamination by pathogens is great challenge in food industry because this is the main cause of food borne illness resulting in severe symptoms as diarrhea that represents a significant hazard. So the most current approach in the world is production of feasible food products to satisfy the consumer's acceptance for palatable and healthy products [1]. LAB represents a group of Gram-positive non-aerobic cocci or rods that can ferment carbohydrates for energy resulting in lactic acid production. These microorganisms are found in different foods of animal and plant sources such as milk, meat, fermented products also fermented vegetables and beverages [2-3]. These bacteria are beneficial in preventing growth of pathogenic microorganisms consequently maintain the nutritive value of food, prolong shelf life and improve quality of fermented products. LAB comprises wide range of genera*:* *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Bifidobacterium, Carnobacterium, Oenococcus, Pediococcus, Leuconostoc* and *Melissococcus* [4]. Interestingly, majority known species are *Lactobacillus acidophilus, L. plantarum, L. casei, L. casei rhamnosus, L. delbrueckii bulgaricus, L. fermentum, L. reuteri, Lactococcus lactis lactis, Lactococcus lactis cremoris, Enterococcus faecalis, Enterococcus faecium, Bifidobacterium bifidum, B. infantis, B. adolecentis, B. longum, B. breve* [5]. LAB has been added as starter cultures in different

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fermented food as fermented dairy and meat products. They enhance fermentation process by its acidification of raw materials by production of organic acids that decrease pH of the food to be unsuitable for food borne pathogens such as *Listeria monocytogenes* [6-7]. LAB induces various antimicrobials metabolites such as hydrogen peroxide lactic acid, carbon dioxide acetic acid and bacteriocins, that have ability to suppress infectious pathogens elongating preservation period and stimulating food products safety [8-12]. Implementation of either LAB producing Bacteriocin or bacteriocins - for food preservation has recognized as great concern [13]. Bacteriocins are ribosomal synthesized antimicrobial peptides [14], so they are labile to protease enzymes in digestive system subsequently efficient in food borne pathogens control and considered safe natural biopreservatives [15]. Also, bacteriocins is an alternative way to standard antimicrobials due to high specificity against pathogens involving multidrug resistant strains but they have some adverse allergic reactions in human [16]. Several schemes have been proposed to classify bacteriocins from Gram-positive bacteria [17] in this classification bacteriocins fall into two classes; lantibiotics (class I) and bacteriocins - containing nonlanthionine (class II). On the contrary, bacteriocins were categorized into four classes [18] through which class III bacteriocins were reclassified as bacteriolysinsas they are lytic enzymes rather than peptides. Currently; circular bacteriocins might be grouped as a different class with further modification [19]. According to biochemical and genetic characteristics [20] two classes of bacteriocins are known; class I- Lantibiotics: they are heat stable peptides, small in size (< 5 kDa) and following translation process typical methyllanthionine and thioether amino acids lanthionine were induced. The most recognized example is nisin [21]. Based on structural similarities, lantibiotic bacteriocins are separated into two subclasses; Subclass Ia includes elongated positive charged peptides that produce cytoplasmic pores in target species membranes. The lantibiotic prototypic nisin is included in this group. Subclass Ib peptides are globular and either negatively charged or without charge. They interfere with reaction enzymes of affected bacteria [22]. Class II, Non-Lantibiotics: these bacteriocins are heat resistance, small in size (<10 kDa) and have mutable molecular mass [23].

The main objectives of this paper were isolation, biochemical and molecular identification using 16S rRNA gene of bacteriocinogenic LAB isolates from fermented dairy products collected from different places in Egypt.

MATERIALS AND METHODS

Isolation, Biochemical and Molecular Identification of LAB Isolates from Dairy Products

Samples Collection: A total number of hundred samples were collected from usual fermented dairy products at different places (Helwan, Maadi, Dokki, Haram and Mohandessen) in Egypt including karish cheese, fermented goat's milk and Laban Rayeb (concentrated sour milk). All sample types were collected in clean sterile bags, then transported to laboratory under complete cooled aseptic conditions and stored at 3 ± 1°C maximum to 24 hours before analysis. The preparation of samples was implemented by homogenization of ten grams of each dairy sample in a stomacher lab-blende mixed with 90 ml sterile sodium citrate solution (2 % w/v) for cheese samples and 90 ml sterile physiological saline (0.85 % NaCl w/v) for fermented milk samples with homogenization for 30 seconds.

Phenotypic Identification of Isolates: - All samples were cultured for 24 hours twice in MRS or M17 broth and identified by morphological, biochemical and physiological tests [24, 25] as follows:

Gram Staining: After Gram staining the under light microscope was used for examination of bacteria.

Catalase Test: 3 % hydrogen peroxide solution was dropped onto 1 ml of freshly prepared isolates liquid cultures incubated overnight of.

CO2 Production from Glucose: 1% fresh cultures isolates incubated overnight was grown in culture tubes containing MRS or M17 broth inverted in Durham tubes supplemented with glucose. The test tubes were incubated for 48-72 hrs at 37°C. Gas production in Durham tubes was an indicator for production of CO2 from glucose.

The identification of LAB was executed based on their morphological, cultural and biochemical criteria as described before [26].

Carbohydrate Fermentation of Isolates:

- The tested carbohydrates were D (+) starch, D (+) amygladin, D (+) melizitose, D (+) sorbitol, D (+) trealose, D (+) arabinose, D (+) raffinose, D (+) Xylose, D (+) ribose, D (+) sucrose, D (+) rhamnose, D (+) cellubiose, D (+) melibiose, D (+) salicin, D (+) mannitol, D (+)mannose, D (+) fructose, D (+) galactose, D (+) glucose, D (+) lactose, D (+)

- Maltose and sterile water were used as positive and negative controls.
- Glycerol culture was stored and kept for further examination at -20°C.

Molecular Characterization of *Lactobacillus* Isolates: The isolates of *Lactobacillus* were genomically characterized using PCR-based assay:

Bacterial DNA Extraction: The bacterial cells growth was carried out in 10 mL MRS broth and incubated at 37°C for 18 hrs. Briefly, A 500 µl aliquot of each culture were added to 500 µl cetyltrimethyl ammonium bromide (CTAB) buffer and incubated at 65 °C for 30 min then centrifuged at 12, 000 *xg* for 10 min. The supernatant was transported to 1.5 mL tube, precipitated with one volume of isopropanol and centrifuged at 12, 000 *xg* for 10 min. After elimination of supernatant, the pellet was rinsed using 500 µl of ethanol 70% v/v for 10 min before drying. Finally, the pellet was stored at -20 °C after dissolved in 100 µl Tris-EDTA buffer [27].

PCR for Identification of the Genus *Lactobacillus* Isolated from Dairy Products: Lactobacillus strains identification was carried out using PCR primers of genus specific designed to the 16S/23S intergenic spacer region ribosomal RNA [28]. The primers sequences were obtained from GenBank sequence database of the National Center for Biotechnology Information. *5' - CTC AAA ACT AAA CAA AGT TTC -3'* was utilized as a forward primer and *5'-CTT GTA CAC ACC GCC CGT CA- 3'* was utilized as a reverse primer. The reaction mixture (50 µL) composed of 2.5 µL of each forward and reverse primers, 4 µL extracted DNA, 25 µL Master Mix (Promega) and 16 µL nuclease free water. The PCR protocol for amplification of target gene: initial denaturation at 95°C for 5 min; 20 cycles of 95°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 30 s (extension); and a final extension step at 72°C for7 min. Agarose gel electrophoresis was applied using 1% agarose. The gel was stained with 0.5 mg/mL ethidium bromide and visualized under UV transilluminator to photograph DNA bands.

Culture Supernatant: Propagation of LAB isolates was applied in 100 ml MRS broth for 16 hours. Cell free solution was obtained by culture centrifugation at 5000 rpm at 4°C for 20 min, then filtration of supernatant using 0.2 ìm, millipore filter. The obtained cell free supernatant was neutralized using 1 N sodium hydroxide to 6.5-7.0 to eliminate the organic acid production effect. Addition of 5 mg. catalase per each one ml of the supernatant and left at 25°C for 30 min to remove the effect of hydrogen peroxide. The supernatants resulting from treatment process were verified for their antimicrobial activities against the indicators bacteria.

Probiotic Properties of Isolates: The bacterial isolates were undergone for some measures such as resistance to low pH, tolerance against bile salt and antimicrobial activity to detect probiotic isolates properties.

Resistance to Low pH: Cells harvested by centrifugation at 5000 rpm for 10min were used with active cultures incubated for 16-18 hours and stored at 4°C. Using phosphate buffer saline (PBS) (PH7.2) washing of pellets was applied one time after that resuspention in PBS (pH 3) and incubated at 37°C. By pour plate technique various bacteria was counted every three hours. Under aerobic conditions proper dilutions were performed and plates were incubated at 37°C for 48 h. besides, growth was inspected by absorbance at optical density (OD) 620.

Growth at Different Temperatures: The MRS and M17 broth media test temperature with indicator bromocresol purple were prepared and transported 5 ml to tubes. After that 0.1 ml overnight cultures were inoculated to tubes and incubated at 10 °C, 15°C, 30°C 37°C and 45 °C for 5 days. The growth of isolates was noticed by color change from purple to yellow during time of incubation at former temperatures [27, 28].

Growth at Different Concentrations of Sodium Chloride: Growth of isolates on broth of MRS or M17 supplied with NaCl at different concentrations (2, 4, 6, 8 and 10 %) was tested. Growth was monitored by measuring OD 620 nm by spectrophotometer (UV-VIS spectrophotometer PD-303 UV Apel Co., LTD Japan) when all test tubes incubated at 37°C for 48-72 hrs.. The MRS or M17 agar was used to measure the viable counts (Log cfu/ml) of isolated strains measured. The viability (%) was calculated as follows: viability (%) = (Log cfu/ml after 24 h / initial Log cfu/ml) × 100 after anaerobically incubation at 37°C for 48 h [29].

Tolerance Against Bile: Active cultures were inoculated with MRS medium containing bile 0.3% for 4 hrs and then incubated for 16-18 hrs. Using pour plate method every viable colonies were counted every hour and growth was monitored by absorbance at OD 620 [30].

Purification of Crude Bacteriocin

Bacteriocin Extraction: Supernatant of cell free culture of *Lactobacillus* producing bacteriocine was obtained by culture centrifugation overnight (20 minutes at 15000 xg at 4°C). 100 mL of culture supernatant was stirred strongly with chloroform (v/v) for 20 minutes and then transferred in separation funnel. The bacteriocin was harvested from the interface layer between the aqueous and organic phases and evaporator concentration device was used for removal of the remaining chloroform.

Salting out Methods for Partially Production of Purified Bacteriocin: *Lactobacillus* producing bacteriocine culture at eighteen hours old was centrifuged at 9000×g for 10 min. To precipitate cell-free supernatant saturated ammonium sulphate 70% was added slowly slowly [31, 32] till the final concentration was 60% (W/V). The mixture was slowly stirred at 4°C for one hour and filtered through cheesecloth. Incubation of the suspension overnight at 4°C and agitation with a magnetic stirrer were conducted. To obtain salt out proteins the suspension was centrifuged at 10000×g for 20 min and dissolved in 10 mM PBS (pH 7.0). The suspension was desalted by dialysis at 4°C during 12h with the same PBS by dialysis cassettes with cut-off of 2000 to 3500. Using regular dialysis bags with cut-off of 10000 -12000 kDa is suitable for this step as the size of most bacteriocins is smaller than 10000 Da. The preparation was concentrated to 5 ml by dialysis against polyethylene glycol and filtered using 0.45 Millipore filter. The activity of bacteriocine was checked for each purification step by well diffusion test against indicator bacteria and partially purified bacteriocin resulted. The molecular weight of derived bacteriocin was estimated in a Tricine SDS-PAGE system [33].

Purification of Bacteriocins by Ion Exchange Chromatography: Ion Exchange Chromatography is used for another purification step to purify partially bacteriocins purified extract. This method used with cation or anion exchange resins that can can separate peptides at definite PH by their electric charge.

Using of cation exchange resins is appropriate way to purify bacteriocin. This method relies on passage bacteriocin extract through the cation exchange column then washed with PBS containing NaCl at 50 to 100 mM concentration to remove anionic residues and the bacteriocin was eluted with a gradient of NaCl from 100 to 1000 mM.

Molecular Weight Determination by Tricine SDS-PAGE: Two gels were prepared, each composed of 5 % stacking gel and 12 % separating gel. 20 ìL of bacteriocin sample aliquot was added to 20 ìL two fold concentrated sample buffer and heated for 5 min at 60 °C. In each gel molecular mass standards polypeptide (94.0 – 14.4 kDa) were used. Electrophoresis at fixed voltage (30 V) was run in the stacking gel and at 150 V during the remaining of separation.

RESULTS AND DISCUSSION

Isolation and Identification of LAB from Dairy Product Samples: The examined samples revealed that a total of 50 isolates identified as LAB recovered from 100 dairy products collected from different markets. The isolates were grouped as 30 were *Lactobacillus* spp., 10 were *Lactococcus* spp. and 10 were *Streptococcus* spp.

Biochemical and Physical Identification of LAB Isolates: Examination of LAB isolates under light microscope showed Gram positive blue-purple color. Biochemical identification indicated negative catalase test with absence of gas bubbles (Table 1). The highest prevalence of LAB isolates was found in karish cheese followed by equal percents in fermented goat milk and Laban Rayed (Table 2). Phonotypical identification of isolates illustrated in (Table 3) showing *Lactobacilli* bacteria (30 isolates) have grown in 4 to 6.5% based on NaCl concentrations. They were homofermentative, or heterofermentive bacilli and all isolates were citrate negative. On the other hand, the coccid LAB (10 isolates) characterized as mesophilic-homofermentative, were belonged to *Lactococcus* spp. while the thermophilic homofermentative characteristic *Streptococcus* spp. (10 isolates) cannot grow at 4 and 6.5% NaCl. Isolated bacteria belonging to *Lactobacillus* spp. characterized by aerobic, Gram-positive bacilli, catalase-negative and homofermentative with yellowish, mucoid, rounded colonies [34, 35]. *Lactobacillus* identified by carbohydrates fermentation pattern growth at15°C, 37°C and 45°C as well as growth at various NaCl concentrations, as described [36] and seen in Tables 1, 3, 4, 5. It could be identified as *Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus lactis*. In accordance with this research, isolation of LAB from dairy products was recorded [33, 34] in which *L. casei* and *L. lactis* were considered as the dominant species in fermented dairy products.

Table 1: Morphological characteristics of isolated LAB strains from dairy product samples

	Lactobacillus (30)	*Streptococcus* (10)	*Lactococcus* (10)
Staining with Gram stain	+	+	+
Arrangement and cell shape	Rods arranged in single, pairs and short, long chains	Cocci arranged in pairs and long chains	Cocci arranged in single, pairs and chains
Production of CO2 from glucose	Homofermentative (-) (20) Heterofermentative (+) (10) *	Homofermentative (-)	Homofermentative (-)
Catalase test	-	-	-
Growth at 15°C	-	-	+
Growth at 37°C	+	+	+
Growth at 45°C	v	+	-
Growth in 4% NaCL	+	-	+
Growth in 6.5% NaCL	+	-	-
Citrate utilization	-	-	-

Table 2: Prevalence of LAB isolates in dairy products

			Lactobacillus isolates		*Lactococcus* isolates		*Streptococcus* isolates	
Type of sample	Number of samples (n = 100)	LAB isolates (n = 50)	No 30	%	No 10	%	No 10	%
Karish cheese,	50	20	15	4.5	5	0.5	6	0.6
Fermented goat's milk	30	10	10	3	3	0.3	2	0.2
Laban Rayeb	20	10	5	1.5	2	0.2	2	0.2

Table 3: Phenotypic and biochemical properties of the isolated lactic acid bacteria obtained from fermented dairy products; fermented goat's milk, Laban Rayeb (concentrated sour milk) and Karish cheese,

						Hydrolysis of		Production of		
Species number	No of isolate	Gas from glucose	Motility	Gram strain	Catalase test	ADH	Citrate	Aesculiun	Acetion	Dextran
1	40	+	-	+	-	-	-	+	-	-
2	15	+	-	+	-	+	-	-	-	-
3	15	+	-	+	-	-	-	+	-	-
4	30	-	-	+	-	+	-	+	-	-

+ : Positive reaction; - : Negative reaction; v: variable. *1. Lactobacillus casei; 2. Lactobacillus fermentum; 3. Lactobacillus plantarum.4.Lactobacillus lactis*

Table 4: Physiological characteristics of the isolates from fermented dairy products (Karish cheese, fermented goat's milk and Laban Rayeb (concentrated sour milk)

		Growth at various temperatures (°C)					Growth at various pH			Growth in the presence of NaC1 (%)				
Species number	Gas production from Glucose	10	15	40	45	50	6.5	9.2	9.6	2	3	4	6.5	10
1	+	-	+	+	-	-	+	-	-	+	+	+	+	-
2	+	-	+	+	-	-	+	-	-	+	+	+	+	-
3	+	-	+	+	-	-	+	-	-	+	+	+	+	-
4	-	-	-	+	-	-	+	+	-	+	+	+	+	-

+: Positive reaction; -: Negative reaction; v: variable. *1. Lactobacillus casei; 2. Lactobacillus fermentum;; 3. Lactobacillus plantarum.*4.*Lactobacilluslactis*

Table 5: Carbohydrate fermentation of lactic acid bacteria isolates obtained from fermented dairy products (Karish cheese, fermented goat's milk and Laban Rayeb (concentrated sour milk)

Specie number	Starch	Amy-gladin	Meli-zitose	Sorb-itol	Trea-lose	Arab-inose	Raff-inose	Xyl-osc	Ribose	Sucrose	Rhamnose	Cellubiose	Melibiose	Salicin	Mannitol	Mannose	Fructose	Galaclose	Glucose	Lactose	Maltose
1	-	+	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-
2	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	+	-	+	-	+
3	-	+	+	+	+	-	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+
4	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	+	+

+: Positive reaction; -: Negative reaction; v: varaible. 1. *Lactobacillus casei*; 2. *Lactobacillus fermentum*; 3. *Lactobacillus plantarum*. 4. *Lactobacilluslactis*

Table 6: Comparative count of *Lactobacillus* using tryptone soya agar and MRS:

Lactobacillus from dairy products	MRS	Tryptone soya agar
Lactobacillus farciminis	5x 10^7	2x 10^7
Lactobacillus curvatus	7x 10^7	6x 10^7
Lactobacillus plantarum	6x 10^7	5x 10^7
Lactobacillus brevis	4x 10^7	3x 10^7
Lactobacillus casei	5x 10^7	2x 10^7

Table 7: Effect of acid on survival of lactobacillus

Lactobacillus from dairy products	Initial count	pH(2) for 2 hours	pH(3) for 2 hours
Lactobacillus farciminis	8.1x 10^7	6.4×10^7	7.89x 10^7
Lactobacillus curvatus	8.3x 10^7	6.2×10^7	8.03x 10^7
Lactobacillus plantarum	8.6x 10^7	6.1×10^7	8.5x 10^7
Lactobacillus brevis	8.6x 10^7	Nil	8.1x 10^7
Lactobacillus casei	8.4x 10^7	6.1×10^7	8.11x 10^7

Table 8: Effect of sodium chloride on survival of *lactobacillus*

Lactobacillus from dairy products	NaCl 2%	NaCl 4%	NaCl 6%	NaCl 8%
Lactobacillus farciminis	+++	++	+	-
Lactobacillus curvatus	+++	+++	-	-
Lactobacillus plantarum	+++	+++	+	-
Lactobacillus brevis	+++	+++	++	-
Lactobacillus casei	+++	+++	+++	+

Molecular Characterization of *Lactobacillus* spp.: Fourteen isolates were confirmed using PCR by amplification of 200 bp PCR products (Fig. 1) recovered from 30 *Lactobacillus* spp. In concurrence with this study combination of both morphological and genomic are required to obtain more precise results. Also, in agreement with this work *Lactobacillus* isolates in fermented dairy products were the most predominant (74%) followed by *Streptococcus* spp. (27 %) and finally *Lactococcus* spp. (14%) [33]. as shown in (Table 2) which revealing that *Lactobacillus* isolates were the most prevalence (15%) followed by equal percent's of both *Lactococcus* and *Streptococcus* spp. (5%) compared with the positive fifty samples obtained in this work.

High growth rate was observed (Table 6), when *Lactobacillus* isolates cultivated in MRS media as it contains enzymatic digests that induce amino acids and nitrogenous substances to support the growth of *Lactobacillus* microorganisms. On the other hand, using whey supplemented with yeast extract for inoculation of *L. reuteri* may increase bacterial population by 5 log cycles [36].

Low growth rate at pH (2) for 2 hours for all isolates was observed using plate count (Table 7) and all isolates were resistant to acid environments at pH(3). *Lactobacillus* casei isolated from dairy products is the most resistant strain for pH (3). In agreement with this study, survival at low pH values of obtained LAB isolates is significant in the selection of probiotic strain [37, 38].

High growth rate was observed using 2% NaCl for all isolates (Table 8) whereas, the rate of growth declined by exaggeration of NaCL concentration till no growing detected for most of isolates at 6 and 8% NaCL and this agrees with Put author name(s) [abdel gawad et., 2010].

Both *Lactobacillus casei* and *Lactobacillus brevis* can withstand bile salt (table 9) as after 2 hours of exposure to bile the growth decreased by 1 log cycle and 2 log cycle. . *Lactobacillus plantarum* withstand bile salts up to 3 hours while *Lactobacillus* curvatus withstand bile up to 1 hour and decline tolerance at 2 and 3 hours.

On contrast, the count of probiotic strains increased after 3 hours incubation with bile salt concentration started with 8.5 x 10^8/ml and ended with 7 x 10^9/ml for *Lactobacillus brevi*s that is mean it can withstand bile salts for one hour and decrease tolerance at two and three hours. This refers to the effect of gastric status on digestion of casein producing amino acids and peptides which promote the growth of probiotic bacteria [40, 41]. Bile salt tolerance of isolated *Lactobacilli* is essential for probiotic strains to colonize in small intestine [42].

Determination of Molecular Size of Bacteriocin: The purified bacteriocin from *Lactobacillus* plantarum revealed a single band at 9 kDa molecular weight using SDS-PAGE (Fig. 2) Similarly, the bacteriocin from *L. saki* ST13BR was 10 kDa molecular weight [43]. The LAB bacteriocins concerning to class-I and II have molecular mass (<5 kDa) and (<10 kDa) respectively.

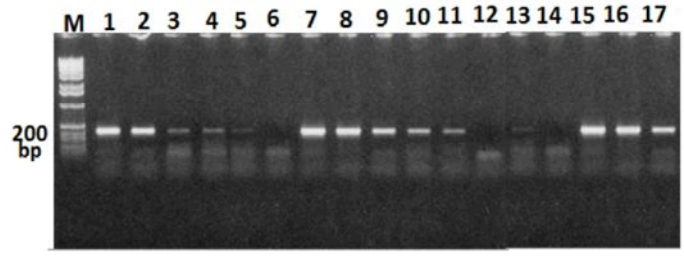

Fig. 1: PCR analysis of some *Lactobacillus* spp. isolates from dairy products. Lane M: 100-bp marker, Lane 1-17: amplification of 200-bp band of *Lactobacillus* spp.

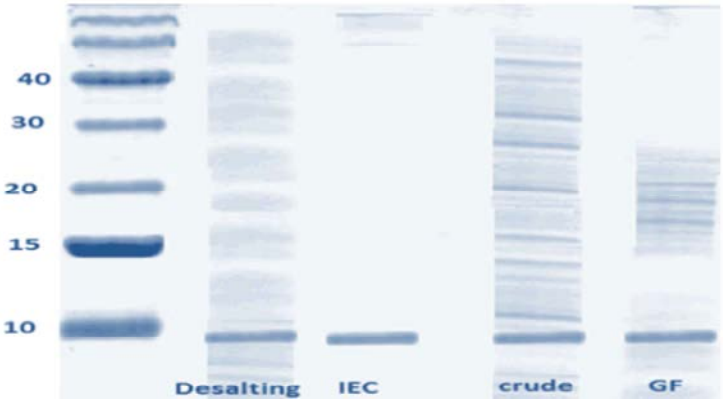

Fig. 2: SDS-PAGE showing; Marker standard pre-stained protein. The SDS-PAGE showed band of purified bacteriocin from *L. plantarum* around 9 kDa molecular weight Gel filtration chromatography (GF) and Ion exchange chromatography (IEC)

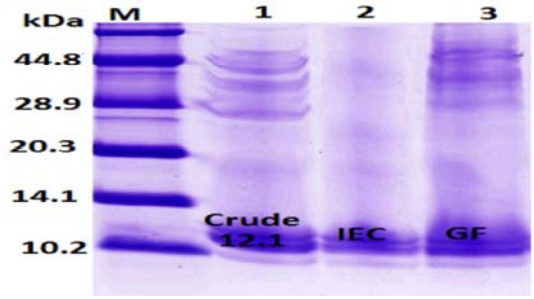

Fig. 3: SDS-PAGE showing; pre-stained protein standard marker. The SDS-PAGE illustrated band of purified bacteriocin from *L. brevis* nearly 12 kDa molecular weight Ion exchange chromatography (IEC) and Gel filtration chromatography (GF)

Table 9: Comparative viability of *Lactobacillus* to bile salts

Lactobacillus from dairy products	0hr	1hr	2hr	3hr
Lactobacillus farciminis	3.5 x10^{10}	3.5 x10^{10}	3 x10^{8}	2.7x10^{8}
Lactobacillus curvatus	8.7x10^{10}	8.5x10^{10}	7.8 x10^{8}	7.5 x10^{8}
Lactobacillus plantarum	6.1 x10^{10}	6.6 x10^{10}	6.2 x10^{8}	6x10^{8}
Lactobacillus brevis	8.5 x10^{10}	7.9 x10^{10}	7.6 x10^{9}	7.0 x10^{9}
Lactobacillus casei	8.8 x10^{10}	8 x10^{10}	7.9x10^{10}	7.1x10^{9}

For example, *L. cin* C-TA33a (4.6 kDa) *L. curvatus* SB13 (10 kDa) and *Pediococcus acidolactici* (3.5 kDa), [44-46]. Purified bacteriocin from *Lactobacillus brevis* showed single band in SDS-PAGE nearly 12. kDa molecular weight (Fig. 3). Bacteriocin laterosporulin produced by *Brevibacillus* sp. strain GI-9 have molecular mass 5.6 kDa [47]. Purification of bacteriocin Bac-GM100 revealed molecular mass 4.375 kDa with monomer protein a [48].

Very low to very high molecular bacteriocin ranging from 2.0 to 94 kDa was detected [49]. In addition, purified *Brevibacillus borstelensis* AG1 bacteriocin, have molecular mass that present on higher side indicating the prospect of new bacteriocin induced from *Brevibacillus borstelensis* AG1 strain [49].

CONCLUSIONS

This research highlighted vast varieties of LAB including lactobacilli in Egyptian dairy products which is the most significant potential probiotics. Besides identification of beneficial natural antimicrobials bacterial communities that can aid in choice of the most efficient preservation method for prolonging the shelf-life of many food products.

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