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Potential Antimicrobial Activities of Probiotic *Lactobacillus* Strains Isolated from Raw Milk

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Abstract

Bacteriocin are highly specific antibacterial proteins which are produced by strains of specific bacteria and showed broad range of antibacterial activity against some mastitis pathogens (*S. aureus*, *E. coli*, *Y. enterocolitica*, *S. uberis* and *S. xylosus*). Bacteriocin producing *Lactobacillus* species were isolated from raw cow, buffalo and, goat milk samples focusing *Lactobacillus* sp isolated from raw cow milk focusing on their safety, antimicrobial properties and further was purified by gel filtration (Sephadex G-100 column). The molecular weight of the purified bacteriocin was varied between 3-30 kDa, showed high thermal stability (up to 100°C) and was active over wide range of pH (3 to 10). The study revealed the possibility of using bacteriocin as producing lactobacilli antimicrobial agent to treatment some mastitis pathogens.

Keywords: Milk; *Lactobacillus* species; Antimicrobial activities; Bacteriocin

Introduction

Lactic acid bacteria (LAB) are Gram positive, non-spore forming, catalase negative cocci or fermentative lactobacilli which produce lactic acid from fermentation of carbohydrates [1,2]. These bacteria are the major component of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal microflora. *Lactobacillus* is one of the most important genera of LAB [3,4]. These organisms are also known to produce various compounds such as bacteriocin which can antagonize the growth of some pathogenic bacteria in foods [5,6]. Lactic acid bacteria are regarded as a major group of probiotic bacteria and have been used successfully to treat acute infantile diarrhea and various diarrheal illnesses [7,8].

They are considered as generally recognized as safe (GRAS) organisms and can be safely used as probiotics for medical and veterinary applications [3,9]. Probiotics are beneficial bacteria as they promote good digestion, boost immune function, increase resistance to infection, inhibit the growth of harmful bacteria and favor the intestinal microflora balance alteration [3,10]. There are also other physiological benefits of probiotics has been published as it help in removal of carcinogens, lowering of cholesterol, immunostimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients, alleviation of lactose intolerance [11].

Another study showed the effects of *Lactobacillus* species on the disease severity, of children with atopic dermatitis and concluded that supplementation of a probiotic is associated with clinical improvement in children with atopic dermatitis [12].

Variety of microorganisms including yeasts, molds and bacteria are present in raw milk. However, among these organisms, only the lactic acid bacteria (LAB) have the property of producing lactic acid from milk sugars by the process of fermentation and thus LAB constitute the predominant microflora of milk [13]. These lactic acid bacteria called probiotic and, it is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. Probiotics are viable lactic acid microorganisms that are believed to provide health benefits when administered in appropriate quantities [14]. The lactic acid bacteria are known to produce antibacterial substances including bacteriocins which can inhibit the growth of several pathogenic bacteria.

Bacteriocins are small ribosomally synthesized peptides that are active against other bacteria and against which the producer has a specific immunity mechanism [15]. Bacteriocins are a heterogeneous group and are usually classified into peptides that undergo significant post-translational modifications (class I) and unmodified peptides (class II) [16]. Bacteriocins from lactic acid bacteria are considered safe additives, useful to control the frequent development of pathogens as broad range of antibacterial activity [17].

These bacteriocins produced by Lactic acid bacteria (LAB) are potent bio-preservative agents and the applications of these in food are currently the subject of extensive research. Finding for new bacteriocins with a wider spectrum of activity and compatibility with different food system is being studied. The present study aimed to investigate the antimicrobial properties of bacteriocins produced by *Lactobacillus* species isolated from raw milk.

Materials and Methods

Isolation of Lactobacillus species

Raw milk of cow, buffaloes, goat and sheep were collected from the local dairy farms of Giza, Egypt during 2011-2013. The samples were collected in a sterile screw cap tube, freshly isolated cultivated on de Man Rogosa Sharpe (MRS) medium and incubated anaerobically at 37°C for 48 hr.

The samples were collected in a sterile screw cap tube and these freshly isolated samples were cultivated on de Man Rogosa Sharpe (MRS) medium (Oxoid, Canada) by incubating anaerobically at 37°C for 48 hr. The suspected colonies were identified on the basis of Gram's staining, catalase test, oxidase, indole, nitrate reduction, and ability to utilize different sugars according to Williams [18]. Finally, the isolates were sub cultured onto MRS agar slants which were incubated at 37°C for 24 h and preserved in 20% glycerol (Oxoid, Canada) at -20°C until further used.

Indicator isolates

The indicator bacteria, *Staphylococcus aureus*, *Escherichia coli*, *S. xylosus*, *S. uberis* and *Yersinia enterocolitica* were kindly supplied from Microbiology Department, Faculty of Veterinary Medicine Cairo University.

Production of crude bacteriocin

Each strain of *Lactobacillus* isolate was propagated in MRS broth (1000 ml) seeded with 10% inoculum (108 cfu/ml) of overnight culture and incubated for 48 h at 150 rpm at 37°C. After incubation, the whole broth was centrifuged at 10,000 × g for 15 min pH values of supernatants were adjusted to pH 6.5-7.0 by the addition of 1N NaOH. The supernatants were membrane filtered (Millipore, 0.22 µm) and stored at 4°C [19]. The cell-free supernatant (crude bacteriocin) was characterized by SDS–PAGE as described by Pot et al. [20].

Purification of bacteriocin

The cell-free culture supernatant (crude bacteriocin) was saturated with 70% ammonium sulfate (Carl Roth, Germany) and kept at 4°C for 4-5 hrs to precipitate out the proteins. After precipitation, the pellet was collected by centrifugation at 10,000 × g for 30 min at 4°C. The pellet was were re-suspended in 25 ml of 0.05 M potassium phosphate buffer (pH 7.0) and dialyzed in a tubular cellulose membrane (Specrapor, 1000 dalton MWco, Fisher Scientific Pittsburgh, PA, USA) against 2 litres of the same buffer at 4°C overnight in spectrapor No. 4 dialysis tubing [19]. The dialyzed protein was applied to a Sephadex G-100 column (1.6 cm × 36 cm) (GE Healthcare, Germany) pre equilibrated with phosphate buffer (pH 7.0). The flow rate was adjusted to 24 ml/h and fractions (1 ml each) were collected in tubes. The collected fractions showing high bacteriocin activity were pooled and lyophilized using Lypholizer (Thermo Fisher Scientific, Germany).

Bacteriocin assay

The antibacterial activity of the bacteriocin extracted from *Lactobacillus* strains was determined using the agar well diffusion method as described by Ivanova et al. [21]. 50 µl of the bacteriocin were placed in 5 mm diameter wells that had been cut in agar plates previously seeded with 106 cfu/ ml of the indicator bacteria. After incubating the plates for 24 hrs at 37°C, the diameter of zone of growth inhibition was measured. Antimicrobial tests were done in duplicate and the mean values were recorded.

Characterization of bacteriocins

Bacteriocins were characterized mainly on the basis of effect of pH and effect of temperature as suggested by Ivanova et al. [21]. The purified bacteriocin from different lactobailli isolates had variable molecular weight (M.Wt).

Effect of pH

To determine effect of pH, 0.5 ml of purified bacteriocin was added into 4.5 ml of nutrient broth at different pH values (3 to 10) and incubated for 30 min at 37°C. Each of the bacteriocin samples treated at different pH values was assayed against indicator bacteria by well diffusion method.

Effect of temperature

Purified bacteriocin (0.5 ml) was added into 4.5 ml of nutrient broth in the test tube. Each test tube was then overlaid with paraffin oil to prevent evaporation and then heated at different temperatures (40, 60, 80 and 100) °C for 10 min. The bacteriocin activity of different heat-treated was measured by well diffusion method.

Results

Identification of Lactobacillus species among the examined milk samples

Different Lactobacilli were isolated from the collected milk samples as shown in Figure 1 and listed in Table 1A and 1B, *L. acidophilus*, *L. salivarius* and *L. delbrueckii* subsp. bulgaricus isolates were specifically detected from cow milk samples. Whereas, *L. acidophilus*, *L. fermentum* and *L. pentosus* isolates were detected from buffalo milk samples. Moreover, *L. acidophilus*, *L. rhamnosus* and *L. delbrueckii* subsp. bulgaricus isolates were detected from ewe milk sample. However, *L. helveticus* and *L. brevis* isolate were detected from goat milk.

Cow isolates	Buffalo isolates	Ewe isolates	Goat isolates
L. acidophilus	*L. fermentum*	*L. rhamnosus*	*L. brevis*
L. salivaruis	*L. acidophilus*	*L. acidophilus*	*L. helveticus*
L. delbrueckii subsp. Bulgaricus	*L. pentosus*	*L. delbrueckii* subsp. Bulgaricus	-

Table 1A: Identification of *Lactobacillus* species among the examined milk samples.

Bacteriocins of *L. delbrueckii* subspecies bulgaricus having M.Wt (16.5 and 21 kDa) isolated from cow and ewe showed inhibition zone against the indicator bacteria. Bacteriocin of *L. brevis* (4.6 kDa) isolated from goat showed inhibition zone against *S. xylosus* and *E. coli* (2 cm and 2.5 cm respectively), while bacteriocin of *L. helveticus* (kDa) showed inhibition zone against *S. aureus* (1.9 cm) and *E. coli* (1.8 cm) (Table 1B).

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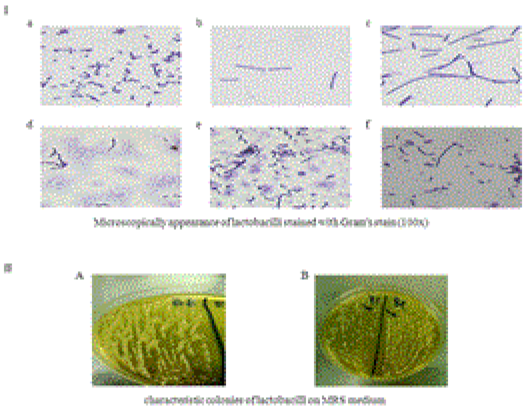

Figure 1: (I) microscopically appearance of lactobacilli stained with Gram's stain (100x). A. L. delberuckii subsp. bulgaricus B. L. brevis C. L. pentosus D. L. salivarius F. L. rhamnosus. (II) characteristic colonies of lactobacilli on MRS medium. A. L. brevis large white colony B. L. acidophilus medium size white colony.

Bacteriocin activity among lactobacilli isolates

Bacteriocin of L. acidophilus of molecular weight (M.Wt=3.5 kDa) isolated from cow had no antibacterial effect on S. xylosus and Yersinia enterocolitica and bacteriocin of L. acidophilus of M.Wt 6.4 kDa isolated from cow had no effect against *Yersinia enterocolitica*. While bacteriocin of *L. acidophilus* of molecular weight 4 kDa isolated from cow inhibit growth of S. xylosus (1.9 cm) and Yersinia enterocolitica (2.5 cm). We also found that bacteriocin of L. pentosus of M.Wt (17 kDa) isolated from buffalo had no significant effect against S. uberis and Yersinia enterocolitica while bacteriocin of L. pentosus of molecular weight 9.8 kDa isolated from buffalo showed zone of inhibition against *S. uberis* (2.1 cm) and *Yersinia enterocolitica* (1.8 cm).

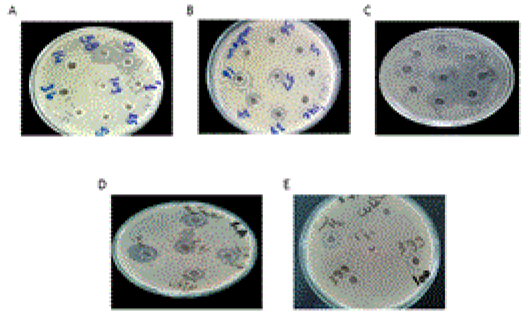

Figure 2: (A) Bacteriocins of *L. acidophilus* against *Yersinia enterocolitica* at 25°C and pH 7. (B) Bacteriocins of *L. salivarius*, *L. pentosus* and *L. acidophilus* against *S. xylosus* at 25°C and pH 7. (C) Bacteriocins of *L. acidophilus* against *S. aureus* at 25°C and pH 7. (D) Bacteriocins of *L. salivarius* and *L. acidophilus* against *S. aureus* at 40°C and pH 3. (E) Bacteriocins of *L. fermentum* against *S. aureus* at 25°C and pH 7. Bacteriocin of *L. fermentum* (10 kDa) has no inhibition effect on *S. aureus* at pH 7 and 25°C, but high inhibition at 80°C and pH 5 was 1.8 cm. Its inhibitory effect on *E. coli* at pH 7 and 25°C was 1.2 cm, which increased to 1.6 cm at 80°C and 1.5 cm at pH 5. The inhibition effect of *S. xylosus* at pH 7 and 25°C was 1.8 cm and no effect at 40°C. No inhibition effect against *Yersinia enterocolitica* at pH 7 and 25°C but at pH 5 and 100°C it was 1.4 cm and 1.5 cm respectively.

Lactobacillus	**SDS kDa**	***S. aureus* (cm)**	***S. xylosus* (cm)**	***S. uberis* (cm)**	***E. coli* (cm)**	***Y. entero-colitica* (cm)**
Cow isolates						
L. acidophilus	7	2	1.3	1.7	2	1.6
L. acidophilus	3.5	1.8	-	2	1.5	-
L. salivarius	15	1.9	2	2.3	1.8	2.5
L. acidophilus	6.4	1.5	1.6	1.8	1.7	-
L. acidophilus	3	1.6	1.5	1.6	1.6	1.6
L. delbrueckii subsp. bulgaricus	16.5	2.5	2.4	1.4	1.6	1.5
L. acidophilus	4	1.5	1.9	1.8	1.5	2.5
Buffalo isolates						
L. fermentum	10	-	1.8	1.4	1.2	-
L. acidophilus	8.3	1.5	1.3	1.2	1.4	1.4
L. pentosus	9.8	2.1	1.8	2.1	1.5	1.8

L. pentosus	17	1.5	1.8	-	1.6	-
L. acidophilus	6.4	1.3	1.2	1.4	2.3	1.4
Ewe isolates						
L. rhamnosus	16.8	1.9	1.6	1.7	1.7	1.7
L. acidophilus	13.75	1.5	-	2	1.6	1.8
L. delbrueckii subsp. bulgaricuss	21	1.7	1.6	-	1.8	1.3
Goat isolates						
L. helveticus	30	1.9	1.7	1.5	1.8	1.7
L. brevis	4.6	1.8	2	2.5	2.4	-

Table 1B: Bacteriocin activity of *Lactobacillus* isolates among the indicator bacteria.

Effect of temperature and pH on bacteriocin activity

The effects of temperature and pH on bacteriocin activity among the examined lactobacilli are well illustrated in Tables 2-5 and Figure 2.

Data shown in Table 2 revealed that the bacteriocin of *L. salivarius* had inhibition effect on *S. aureus* (1.9 cm) at pH 7 and 25°C which increased to 2.6 cm at both pH 3 and 80°C. The bacteriocin had inhibition effect on *S. uberis* at pH 7 and 25°C reached to 2.3 cm. No increase of the zone of inhibition with increase of temperature but slightly increased at pH 3 (2.4 cm) was recorded. The bacteriocin has no effect on all pathogens at pH 10.

Table 3 illustrated that bacteriocin of *L. acidophilus* (8.3 kDa) had inhibition effect to *S. aureus* at pH 7 and 25°C (1.5 cm) and increased at 60°C to 1.8 cm and at pH 3 to 2.5 cm. Also in case of *S. xylosus* and *S. uberis* the inhibition zone increased from 1.3 cm and 1.2 cm at pH 7 and 25°C to 1.6 cm and 2.7 cm; 1.8 cm and 2.7 cm respectively at 60°C and pH 3. Inhibition zone against *E. coli* and *Yersinia enterocolitica* increased at 100°C and pH 3.

***L. fermentum* (10 kDa)**	***S. aureus* (cm)**	***E. coli* (cm)**	***S. uberis* (cm)**	***S. xylosus* (cm)**	***Y. entero-colitica* (cm)**
Bacteriocin activity measured					
At 25°C and pH 7	-	1.2	1.4	1.8	-
Activity at different temperature with same pH 6.5					
40	1.4	1.3	1.2	-	1.2
60	1.5	1.5	1.7	1.4	1.4
80	1.8	1.6	1.2	1.5	1.2
100	1.2	1.5	2	1.3	1.5
Activity at different pH with constant temperature at 37°C					
3	1.7	1.4	1.2	1.2	1.3
5	1.8	1.5	1.3	1.5	1.4
8	1.5	-	-	1.2	-
10	-	-	1.2	-	-
***L. salivarius* (15 kDa)**	***S. aureus* (cm)**	***E. coli* (cm)**	***S. uberis* (cm)**	***S. xylosus* (cm)**	***Y. entero-colitica* (cm)**
Bacteriocin activity measured					
At 25°C and pH 7	1.9	2	2.3	1.5	2.5
Activity at different temperature with same pH 6.5					
40	1.2	2.6	1.9	1.7	2.6
60	1.8	1.7	1.3	1.7	1.7
80	2.6	1.2	2.1	1.2	1.4
100	2.1	-	2	-	1.2
Activity at different pH with constant temperature at 37°C					
5	1.7	1.2	1.2	1.4	1.2
3	2.6	2.4	2.4	2.4	1.6
8	-	1.6	-	1.6	-
10	-	-	-	-	-

Table 2: Effect of temperature and pH on bacteriocin activity of *L. fermentum* (10 kDa) and L. salivarius (15 kDa) from cow isolates.

As shown in Table 4 showed, bacteriocin of *L. acidophilus* had inhibition effect to *S. aureus* at pH 7 and 25°C reached to 2 cm, increased at 40°C and pH 5 to 2.2 and 2.5 cm respectively. While no inhibition effect of the bacteriocin on *S. xylosus* at pH 7 and 25°C, but zone of inhibition reached to 1.6 cm at 60°C and 1.7 cm at pH, 8 and 10 each.

L. acidophilus **(8.3 kDa) buffalo isolate**	***S. aureus*** **(cm)**	***S. xylosus*** **(cm)**	***E. coli*** **(cm)**	***S. uberis*** **(cm)**	***Y. entero-colitica*** **(cm)**
Bacteriocin activity measured					
At 25°C and pH 7	1.5	1.3	1.4	1.2	1.4
Activity at different temperature with same pH 6.5					
40	1.7	1.5	1.6	1.7	1.5
60	1.8	1.6	1.8	1.8	1.6
80	1.6	1.4	1.3	1.4	1.5
100	1.3	1.5	1.9	1.3	1.7
Activity at different pH with constant temperature at 37°C					
3	2.5	2.7	1.8	2.7	2.5
5	2	2.5	1.6	1.4	1.6
8	2	1.3	1.2	2.2	1.5
10	1.7	2	1.7	-	1.3
L. acidophilous **(13.75 kDa) Ewe isolate**	***S. aureus*** **(cm)**	***S. xylosus*** **(cm)**	***E. coli*** **(cm)**	***S. uberis*** **(cm)**	***Y. entero-colitica*** **(cm)**
Bacteriocin activity measured					
At 25°C and pH 7	2	-	1.6	2	1.8
Activity at different temperature with same pH 6.5					
40	2.2	1.5	1.8	1.9	1.7
60	1.9	1.6	1.7	2.3	2.1
80	1.5	1.4	1.6	2.5	2.3
100	1.2	1.3	1.1	2.6	2.1
Activity at different pH with constant temperature at 37°C					
5	2.5	1.7	2.8	2.2	2.6
3	2.1	1.5	2.7	2	2.3
8	1.5	1.7	1.8	1.7	2
10	1.4	1.7	1.8	2	1.5

Table 3: Effect of temperature and pH on bacteriocin activity of *L. acidophilus* (8.3 kDa and 13.75 kDa) from buffalo and ewe isolates.

The inhibition effect of bacteriocin on *S. uberis* increased from 2 cm at pH 7 and 25°C to 2.6 cm at 100°C and 2.2 cm at pH 5. The inhibition effect of bacteriocin on *E. coli* and *Yersinia enterocolitica* increased at pH 5 but different in temperature, *E. coli* increased diameter of zone of inhibition at 40°C but *Yersinia enterocolitica* at 80°C.

The bacteriocin of *L. delbrueckii* subsp. bulgaricus had inhibition effect of *S. aureus* 1.7 cm at 25°C and pH 7 and no increase in inhibition zone by increase temperature but increased to 2.8 and 2.7 cm at pH 5 and 3 respectively. The bacteriocin has inhibition effect to *S. xylosus* and *E. coli* at 40°C (2 and 2.3 cm) and pH 5 (2.9 and 1.9 cm) respectively. And had inhibition effect to S. uberis and *Yersinia enterocolitica* at 80°C (2 and 1.7 cm) and pH 5 (2.9 and 1.8 cm) respectively (Table 4).

Table 4 concluded that the bacteriocin of *L. rhamnosus* had inhibition effect on *S. aureus*, *S. xylosus*, *E. coli* and *Yersinia enterocolitica* at pH 7 and 25°C with inhibition zone ranged from 1.6 to 1.9 cm. There was no increase of inhibition zone by increase temperature while it increased at pH 3. The bacteriocin of *L. rhamnosus* has no effect on pH 10.

***L. delbrueckii* subsp. bulgaricus (21 kDa)**	***S. aureus* (cm)**	***S. xylosus* (cm)**	***S. uberis* (cm)**	***E. coli* (cm)**	***Y. entero-colitica* (cm)**
Bacteriocin activity measured					
At 25°C and pH 7	1.7	1.6	-	1.8	1.3
Activity at different temperature with same pH 6.5					
40	1.4	2	1.2	2.3	1.6
60	1.4	1.7	1.5	1.2	1.5
80	1.7	1.8	2	1.9	1.7
100	1.7	1.5	1.7	1.9	1.5
Activity at different pH with constant temperature at 37°C					
3	2.8	2.9	2.9	1.9	1.8
5	2.7	2.6	2.6	1.7	1.5
8	1.7	1.6	1.5	1.4	1.2
10	2.3	1.7	2.9	1.8	1.5
***L. rhamnosus* (16.8 kDa)**	***S. aureus* (cm)**	***S. xylosus* (cm)**	***S. uberis* (cm)**	***E. coli* (cm)**	***Y. entero-colitica* (cm)**
Bacteriocin activity measured					
At 25°C and pH 7	1.9	1.6	1.7	1.7	1.7
Activity at different temperature with same pH 6.5					
40	1.5	1.4	1.6	1.5	1.3
60	1.8	1.1	1.7	1.5	1.3
80	1.7	1.1	1.6	1.3	1.1
100	1.9	1.2	1.8	-	1.2
Activity at different pH with constant temperature at 37°C					
5	2.3	1.5	2.7	1.7	1.5
3	2.2	1.7	2.7	2.5	2.3
8	1.5	-	1.8	1.7	1.5
10	-	-	-	-	-

Table 4: Effect of temperature and pH on bacteriocin activity of *L. delbrueckii* subsp. bulgaricus (21 kDa) and *L. rhamnosus* (16.8 kDa) from Ewe isolates.

Another set of data revealed that the bacteriocin of *L. brevis* had inhibition effect on *S. aureus* 1.8 cm at pH 7 and 25°C increased to 2.3 cm at 80°C and at pH 5 and 10 has inhibition zone 2.5 and 2.4 cm respectively, while no effect at pH 8. The bacteriocin has inhibition effect on *S. xylosus* 2 cm at pH 7 and 25°C then increased to 2.3 cm at 80°C and pH 5 but had no effect at pH 8. While no increased in zone of inhibition against *S. uberis* and *E. coli* by increased temperature and change in pH (Table 5).

***L. brevis* (4.6 kDa)**	***S. aureus* (cm)**	***S. xylosus* (cm)**	***S. uberis* (cm)**	***E. coli* (cm)**
Bacteriocin activity measured				
At 25°C and pH 7	1.8	2	2.5	2.4
Activity at different temperature with same pH 6.5				
40	2	1.8	2.3	2.1
60	2.2	1.9	1.8	1.8
80	2.3	2.3	1.6	1.5
100	1.5	1.5	1.2	1.4
Activity at different pH with constant temperature at 37°C				
3	2.5	2.3	1.9	1.7
5	2.2	2	1.7	1.2
8	-	-	-	1.5
10	2.4	1.5	2	1.5

Table 5: Effect of temperature and pH on bacteriocin activity of *L. brevis* (4.6 kDa) from Goat isolates.

Discussion

Milk and milk products are usually associated with probiotic bacteria, which provide supplements in maintaining beneficial intestinal balance [22]. Lactobacilli (LAB) which can produce antibacterial agent against some major food spoilage and pathogenic bacteria are present in fresh cow milk, fermenting corn slurry and the feces of human neonates, pig and albino rat [23]. There were different bacilli species present in milk of cows, buffalo, ewe and goat which contain bacteriocin. These different bacteriocins have different impact on zone of inhibition which is dependent on temperature and pH.

The purified bacteriocin from different *Lactobacillus* isolates has wide range of variable molecular weight (3-30 kDa). Another finding reported the molecular weight of the bacteriocin from *L. plantarum* ST13BR as 10 kDa [24]. The bacteriocins of lactic acid bacteria belonging to class-I and II have molecular weight <5 kDa and <10 kDa respectively [25].

Various *Lactobacillus* species have been evaluated for the prevention or treatment of various infectious diseases and these were found to be safe [22]. *L. acidophilus* and *L. rhamnosus* showed high activity against the most predominant bacteria which were isolated from cases suffering from ovarian inactivity [26].

Bacteriocins are used to inhibit the growth of pathogenic organisms in foods [27]. All the lactic acid bacteria screened for bacteriocin production, *Lactobacillus bulgaricus*, *L. lactis*, *L. acidophilus*, *Lactococcus lactis*, *Streptococcus thermophilus, S. cremoris*, *Pediococcus halophilus* and *P. cerevisiae* produced bacteriocin activity between 4800 and 6000 Au/ml against *Staphylococcus*, *Salmonella*, *Bacillus*, *Shigella* and *Pseudomonas* species [28]. This property of inhibition made them potent bacteriocin producers.

The bacteriocin producing strain was isolated from bovine and ovine milk samples and the selected strain was identified. The susceptibilities of the examined Gram-positive (*S. aureus*, *S. xylosus* and *S. uberis*) and Gram-negative bacteria (*E. coli* and *Y. enterocolitica*) to growth inhibition by the bacteriocin of *Lactobacillus* species were recorded. Gilliland and Speck [29] reported that lactobacilli showed stronger antibacterial effect against Gram positive than Gram negative bacteria. It is clear that bacteriocin of the examined isolates shows antibacterial activity against all examined indicator bacteria except *L. acidophilus* bacteriocin (3.5 kDa) which had no antibacterial effect on *S. xylosus* and *Yersinia enterocolitica* isolates.

Interestingly, some bacteriocins from differet isolates have no effect on specific bacteria likewise bacteriocins of *L. acidophilus* (6.4 kDa) and *L. brevis* (4.6 kDa) had no effect against *Yersinia enterocolitica* whereas Bacteriocin of *L. pentosus* (17 kDa) had no effect against *S. uberis* and Yersinia enterocolitica. Also, bacteriocin of *L. acidophilus* (13.75 kDa) had no effect against *S. xylosus*, while bacteriocin of *L. delbrueckii* subsp. bulgaricus (21 kDa) had no effect against *S. uberis* and bacteriocin of *L. fermentum* (10 kDa) had no effect against *S. aureus* and *Yersinia enterocolitica*.

Similarly, bacteriocin from *L. plantarum* was found to be active against pathogenic bacteria including *Cl. sporogenes*, *E. faecalis*, *E. coli* and *S. aureus* [30,31]. Antibacterial activity of bacteriocin produced by isolated probiotics showed that, *L. rhamnosus* and *L. plantarum* had strong antibacterial effect against enteric bacterial pathogens [22].

L. Plantarum and *L. rhamnosus* from goat and *L. plantarum* from cow milk were more effect probiotic [22]. Lengkey and Adriani [32] showed the results about the sensitivity of the lactic acid bacteria on *P. aeruginosa* and *S. aureus*. Jin et al. [33] had earlier reported the inhibition of *E. coli* and Salmonella strains by *Lactobacillus* species from chicken intestine. Ehrmann et al. [34] reported the inhibition of fecal strains (*E. coli, S. Enteritidis and S. Typhimurium*) by lactobacilli isolated from crops and intestine of ducks.

There is also reported the inhibition of food borne bacteria by bacteriocins from *L. gasseri* [35]. They observed that several strains of *L. gasseri* showed wide inhibitory activity against *L. monocytogenes*, *Bacillus cereus*, *S. aureus*, and *E. coli*. Recently Heredia-Castro et al. [36] recorded that *Lactobacillus* species from cheese were shown to produce bacteriocin-like substances active against *S. aureus*, *L. innocua, E. coli* and *S. Typhimurium* by using the disk diffusion method. Bacteriocin was active in a wide range of pH, but the maximum activity was observed at pH 3 and pH 5. Bacteriocins produced by *L. plantarum* and *L. brevis* retained their antimicrobial activity in an acidic pH range of 2.0 to 6.0, while inactivation occurred at pH 8.0 to 12.0 [37]. The present data revealed that most of the extracted bacteriocins of *Lactobacillus* species were stable in acidic as well as alkaline pH (3 to 10) except *L. fermentum* (10 kDa), *L. rhamnosus* (16.8 kDa) and *L. salivarius* (15 kDa) at pH 10.

The bacteriocins of *L. acidophilus* and *L. bulgaricus* were stable between pH 3 and pH 10 while *L. helveticus* was found to be sensitive to pH 10 [38]. Another study showed that bacteriocins of vaginal lactobacilli were stable at pH 4.5 to 7 but sensitive to pH 9 [39]. Bacteriocin-like substances produced by *Lactobacillus* strain showed potential for application as a food bio-preservative [36].

The effect of temperature on bacteriocin activity in terms of inhibition zones was study. Bacteriocins of all the selected *Lactobacillus* species were stable up to 100°C. It has been found to be thermostable in nature as it can withstand high temperature up to 100°C, although a partial loss in the activity was observed with a continuous increase in temperature. Thermo stability of bacteriocin at high temperature makes it possible to sterilize the food products even at room temperature. Earlier studies revealed that bacteriocins produced by *L. para casei, L. lactis, L. plantarum* and *L. pentosus* remained active after heating till 121°C for 20 min [40].

Conclusion

Bovine and ovine milk are conceder as a source of bacteriocin producers. *Lactobacillus* species could be of great interest in the production of bio preservatives for the food industries. The present study revealed that bacteriocins of *Lactobacillus* species from milk were stable at temperature up to 80°C and between pH 3 to 8 had strong antibacterial against many bacterial pathogens. Bacteriocin producers are recommended to food processing industries to enhance extension of shelf life of food products.

Conflict of interest

The authors declare that there is no conflict of interests.

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References

1. Aasen IM, Møretrø T, Katla T, Axelsson L, Storrø I, et al. (2000) Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. Appl Microbiol Biotechnol 53: 159-166.
2. Suskovic B, Kos J, Goreta S (2001) Role of Lactic Acid Bacteria and Bifidobacteria in Synbiotic Effect. Food Technol. Biotechnol 39: 227-235.
3. Djomne Victor Sieladie, Ngoufack François Zambou, Pierre Marie Kaktcham, Alberto Cresci, Florence Fonteh (2011) Probiotic properties of

lactobacilli strains isolated from raw cow milk in the western highlands of Cameroon. Innovative Romanian Food Biotechnology 9: 12-28.

4. Coeuret V, Dubernet S, Bernardeau M, Gueguen M, Vernoux JP, et al. (2003) Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Lait 83: 269–306.
5. Oyetayo VO, Adetuyi FC, Akinyosoye FA (2003) Safety and protective effect of *Lactobacillus acidophilus* and *Lactobacillus casei* used as probiotic agent in vivo. Afr J Biotech 2: 448-452.
6. Abdelbasset M, Djamila K (2008) Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk Raïb. Afr J Biotechnol 7: 2908-2914.
7. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL, et al. (1995) Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr 21: 224-226.
8. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, et al. (2006) Probiotic potential of *Lactobacillus* strains isolated from dairy products. International Dairy Journal 16: 189-199.
9. Fuller R (1989) Probiotic in man and animals. A Review. Journal of Applied Bacteriology 90: 3452- 3453.
10. Helland MH, Wicklund T, Narvhus JA (2004) Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. Int J Food Microbiol 91: 305-313.
11. Parvez S, Malik KA, Ah Kang S, Kim HY (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100: 1171-1185.
12. Wang IJ, Wang JY (2015) Children with atopic dermatitis show clinical improvement after *Lactobacillus* exposure. Clin Exp Allergy 45: 779-787.
13. Ogier JC, Son O, Gruss A, Tailliez P, Delacroix-Buchet A, et al. (2002) Identification of bacterial microflora in dairy products by temporal temperature gradient gel electrophoresis. J Appl Environ Microbiol 68: 3691-3701.
14. Rijkers GT, de Vos WM, Brummer RJ, Morelli L, Corthier G, et al. (2011) Health benefits and health claims of probiotics: bridging science and marketing. Br J Nutr 106: 1291-1296.
15. Svetoch EA, Stern NJ (2010) Bacteriocins to control Campylobacter spp. in poultry-A review. Poult Sci 89: 1763-1768.
16. Cotter PD, Ross RP, Hill C (2013) Bacteriocins-a viable alternative to antibiotics? Nat Rev Microbiol 11: 95-105.
17. Parada JL, Caron CR, Medeiros ABP, Soccol CR (2007) Bacteriocins from lactic acid bacteria: purification, properties and use as biopreservatives. Braz arch biol technol 50: 512-542.
18. Williams ST (1989) Bergeys manual of systematic bacteriology. Vol. 4. Baltimore: Williams and Wilkins.
19. Ogunbanwo ST, Sanni AI, Onilude AA (2003) Characterization of bacteriocin produced by *Lactobacillus plantarum* F and *Lactobacillus brevis* OGI. African J Biotechnol 2: 219-227.
20. Pot B, Vandamme P, Kersters K (1994) Chemical Methods in Prokaryotic Systematics. In Modern Microbiological Methods series 5. pp: 493-521.
21. Ivanova I, Kabadjova P, Pantev A, Danova S, Dousset X (2000) Detection, purification and partial characterization of a novel bacteriocin substance produced by *Lactococcus lactis* subsp. lactis b14 isolated from Boza-Bulgarian traditional cereal beverage. Biocatalysis 41: 47-53.
22. Tambekar DH, Bhutada SA (2010) An evaluation of probiotic potential of Lactobacillus sp. from milk of domestic animals and commercial available probiotic preparations in prevention of enteric bacterial infections. Recent Research in Science and Technology 2: 82-88.
23. Oyetayo VO (2004) Phenotypic characterisation and assessment of the inhibitory potential of *Lactobacillus* isolates from different sources African Journal of Biotechnology 3: 355-357.
24. Todorov SD, van Reenen CA, Dicks LM (2004) Optimization of bacteriocin production by *Lactobacillus plantarum* ST13BR, a strain isolated from barley beer. J Gen Appl Microbiol 50: 149-157.
25. Jeevaratnam K, Jamuna M, Bawa AS (2005) Biological preservation of foods-bacteriocins of lactic acid bacteria. Indian J Biotechnol 4: 446-454.
26. Abd El-Moez SI, Ahmed WM, El-Jakee JA, El-Seedy FR (2008) Observations on *Lactobacillus* spp. In the Genital Tract of Buffalo-Cows with Emphasis on its In Vitro Probiotic Activity. Global Veterinaria 2: 15-21.
27. Shih-Chun Yang, Chih-Hung Lin, Calvin T. Sung, Jia-You Fang (2014) Antibacterial activities of bacteriocins: application in foods and pharmaceuticals. Front Microbiol 5: 241.
28. Mohammed SD, Ijah UJJ (2013) Isolation and screening of lactic acid bacteria from fermented milk products for bacteriocin production. Annals Food Science and Technology 14: 122-128.
29. Gilliland SF, Speck ML (1977) Antagonistic action of *Lactobacillus* acidophilus towards intestinal and food pathogen in associative culture. J Food Protect 40: 820-823.
30. Aly EA (2007) Characterization of a bacteriocin-like inhibitory substance produced by *Lactobacillus plantarum* isolated from Egyptian home-made yogurt. Science Asia 33: 313-319.
31. Atta HM, Refaat BM, El-Waseif AA (2009) Application of biotechnology for production, purification and characterization of peptide antibiotic produced by probiotic *Lactobacillus plantarum* NRRL B-227. Global J Biotechnol Biochem 4: 115-125.
32. Lengkey HAW, Adriani L (2009) Effects OF milk fermented with *Lactobacillus acidophilus* and Bifidobacterium spp., on lactic acid and acetic acid content and on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Biotechnology in Animal Husbandry 25: 719-724.
33. Jin LZ, Ho YW, Abdullah N, Ali MA, Jalaludin S (1996) Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. Lett Appl Microbiol 23: 67-71.
34. Ehrmann MA, Kurzak P, Bauer J, Vogel RF (2002) Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. J Appl Microbiol 92: 966-975.
35. Itoh T, Fujimoto Y, Kawai Y, Toba T, Saito T (1995) Inhibition of food-borne pathogenic bacteria by bacteriocins from *Lactobacillus gasseri*. Lett Appl Microbiol 21: 137-141.
36. Heredia-Castro PY, Méndez-Romero JI, Hernández-Mendoza A, Acedo-Félix E, González-Córdova AF, et al. (2015) Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances produced by *Lactobacillus* spp. isolated from artisanal Mexican cheese. J Dairy Sci 98: 8285-8293.
37. Bromberg R, Moreno I, Zaganini CL, Delboni RR, Oliveira JD, et al. (2004) Isolation of bacteriocin producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. Braz J Microbiol 35: 137-144.
38. Moghaddam MZ, Sattari M, Mobarez AM, Doctorzadeh F (2006) Inhibitory effect of yogurt Lactobacilli bacteriocins on growth and verotoxins production of Enterohemorrhgic Escherichia coli O157:H7. Pak J Biol Sci 9: 2112-2116.
39. Alpay S, Aydin F, Kilich SS (2003) Antimicrobial activity and characteristics of bacteriocins produced by vaginal Lactobacilli. Turk J Med Sci 33: 7-13.
40. Papanthanasopoulos MA, Krier F, Junelles AMR, Lefebvre G, Le Caer JP, et al. (1997) Multiple bacteriocin production by Leuconostoc mesenteroides TA33a and other Leuconostoc/Weissella strains. Current Microbiol 35: 331-335.