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Lactococcus lactis subsp. lactis isolated from fermented milk products and its antimicrobial potential

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ABSTRACT

The fermented milk samples (n = 25) were examined for the presence of lactic acid bacteria (LAB) with broad antimicrobial potential. Isolates were identified based on biochemical profiling. Antibacterial activity of the LAB was determined against *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*. *L. lactis* subsp. *lactis* showed broad antimicrobial spectrum compared to other isolates and probiotic evaluation showed viability of *L. lactis* at low pH (3), 3% and 0.3% bile salts. Bacteriocin from the LAB isolate (*L. lactis* subsp. *lactis*) was partially purified by precipitation, dialysis and microfiltration followed by molecular weight determination. The partially purified bacteriocin was used for biopreservation of poultry meat against target bacteria (*S. aureus* and *S. typhimurium*). The antimicrobial metabolites were also found active against *S. typhimurium* (11.2 ± 1.72 mm) and *E. coli* (13.4 ± 1.15 mm). LAB reduced the number of target bacteria by 10-fold in milk after 24 h of incubation. Crude bacteriocin reduced the number of target bacteria in poultry meat, from initial count of 10⁵–10⁶ CFU/g to 10¹ CFU/g for *S. aureus* and to 10² CFU/g for *S. typhimurium* respectively.

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Patógenos transmitidos por los alimentos; Seguridad alimenticia; Bacteriocina; Bacterias de ácido láctico; Estudio de cultivo protegido

Lactococcus lactis subsp. lactis aislada de productos lácteos fermentados y su potencial antimicrobiano

RESUMEN

Se examinaron muestras de leche fermentada (n = 25) en busca de bacterias de ácido láctico (LAB) con amplio potencial antimicrobiano. Los aislados se identificaron a partir de perfiles bioquímicos utilizando kits API. Al respecto, se comprobó la actividad antibacteriana de las LAB contra *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* y *Listeria monocytogenes*. El aislado bacteriano de *L. lactis* subsp. *lactis* mostró tener un amplio espectro antimicrobiano en comparación con otros aislados. Asimismo, la evaluación de probióticos dio cuenta de la viabilidad de *L. lactis* a pH bajo (3), concentración de sal (NaCl) al 3% y de sales biliares al 0.3%. Posteriormente, se purificó parcialmente la bacteriocina del aislado de LAB (*L. lactis* subsp. *lactis*) empleando precipitación, diálisis y microfiltración, determinándose luego su peso molecular. La bacteriocina parcialmente purificada se utilizó para la conservación biológica de carne de aves de corral contra bacterias diana (*S. aureus* y *S. typhimurium*). Además, se constató la actividad de los metabolitos antimicrobianos contra *S. typhimurium* (11.2 ± 1.72 mm) y *E. coli* (13.4 ± 1.15 mm). Se comprobó que, después de 24 horas de incubación, las LAB redujeron en 10 veces la cantidad de bacterias diana en la leche. La bacteriocina en bruto redujo el número de bacterias diana en la carne de ave, que del recuento inicial de 10⁵–10⁶ CFU/g pasaron a 10¹ CFU/g para *S. aureus* y hasta 10² CFU/g para *S. typhimurium*.

1. Introduction

Probiotics are nonpathogenic microbes, which induce positive health benefits on the host when ingested in adequate amount (FAO/WHO, 2006). The probiotic intake has been found helpful in reducing various ailments ranging from diarrhea to cancer. The probiotics also help in lactose intolerance, lower cholesterol level and enhance the nutrients utilization (Angmo, Kumari, & Bhalla, 2016). Lactic acid bacteria (LAB) play an

important role in animal and human health by dominating and balancing the gut microflora. LAB exhibit antagonistic activity against pathogenic bacteria and inhibit the pathogens and spoilage microorganisms in food and food products (Ramirez-Chavarin, Wachter, Eslava-Campos, & Perez-Chabela, 2013). The strains of *Lactococcus*, *Lactobacillus*, and *Pediococcus* show good viability and lowering of pH in fermented foods, due to which they are predominantly used as a starter culture (Reis, Paula, Casarotti, & Penna, 2012; Todorov et al., 2017).

Organic acids, hydrogen peroxide, bacteriocin and other metabolites produced by LAB, play a key role in their antimicrobial capabilities (Branco et al., 2010). The rising concern for human well-being and healthy food urges the scientists to explore novel and safe antibacterial compounds for food preservation. The synthetic and chemical preservatives are associated with health hazards therefore, the natural antimicrobials have always been preferred as food preservatives (Kaškonienė et al., 2017; Sadiq, Tarning, Aye Cho, & Anal, 2017).

Fermented foods are consumed worldwide, due to their health benefits and cultural importance. The desired microbes associated with fermented foods are beneficial for health and food preservation. In Asia, a wide variety of traditional fermented foods are consumed and these traditional fermented foods can be explored for the isolation of probiotics with preservation potential (Angmo et al., 2016). *Lactococcus lactis* has been recognized as generally regarded as safe (GRAS) by United States Food and Drug Administration (USFDA) and its antimicrobial metabolites, particularly nisin, are in use for the control of spoilage bacteria and foodborne pathogens in food (Akbar & Anal, 2014a). Bacteriocin is an effective substitute of chemical preservatives in the food industry. The bacteriocin-producing probiotics have an advantage in competitive interactions with the pathogenic bacteria from the food matrix (Todorov et al., 2017). LAB and their metabolites provide health and nutritional benefits to consumers. Presence of LAB in fermented milk products is not only the source of antimicrobials but also provides flavoring compounds such as acetaldehyde in yoghurt and cheese (Parvez, Malik, Ah Kang, & Kim, 2006).

Spoilage bacteria and foodborne pathogens are of great concern for the food industry and consumers (Akbar & Anal, 2015, 2011; Sadiq, Hanpithakpong, Tarning, & Anal, 2015). Approximately, 22.8 million cases of foodborne illnesses are recorded every year in South East Asia with a death toll of 27,600 (Akbar & Anal, 2013). Use of LAB for the bio-control of pathogens and food preservation is getting attention due to their additional health benefits apart from preservation potential and safe status (Kumaree, Akbar, & Anal, 2015; Reis et al., 2012). The use of LAB and its bacteriocin in a combination with other preservatives is commercially applicable in hurdle technology for shelf life extension of food (Jofre et al., 2008). The aim of this study was to isolate the bacteriocinogenic *L. lactis* subsp. *lactis* from fermented milk products and to evaluate its antimicrobial and probiotic characteristics. The LAB was further evaluated for its preservation potential against foodborne bacteria in poultry meat and cow milk samples.

2. Materials and methods

2.1. Isolation and identification of lactic acid bacteria

The local fermented milk products ($n = 25$) were collected from the greater Bangkok region of Thailand aseptically in sterile sample bottles and transported to Biotechnology Laboratory of the Asian Institute of Technology Thailand. The local fermented milk products were further characterized into yogurt, lassi, cheese, cultured buttermilk, flavored fermented milk and five samples of each fermented milk product were collected. The isolation and identification of LAB were carried out by using the methods of Kumaree et al.

(2015) and Ghatani and Tamang (2017) with slight modifications. Briefly, 10 mL of the sample was mixed aseptically with 90 mL of sterile normal saline (NaCl, 0.85% w/v) and further diluted serially up to 10^{-7} . The diluted sample (0.1 mL) was spread over de Man-Rogosa-Sharpe (MRS) agar (Himedia India) supplemented with bromocresol purple (0.06%, w/v) and incubated anaerobically at 37°C for 48–72 h. The isolates were purified by sub-culturing on MRS agar and identified initially with the help of Gram staining (cell morphology and arrangement) and catalase activity (3% H_2O_2). The isolates were then finally confirmed by biochemical profiling using API kits (BioMérieux, Marcy l'Étoile, France) (Supplementary material, Table S2-S3).

2.2. Screening of antagonistic activity

Four prominent foodborne pathogens including two Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and two Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) bacteria were used to study the inhibitory action of the isolated LAB. Antibacterial activity was determined against the selected foodborne pathogens using the spot on lawn, agar well and mix culture methods following Akbar and Anal (2014a). In the spot on lawn method, 10 μ l of fresh LAB culture was spotted on the surface of dry MRS agar and incubated at 37°C for 24–48 h. Molten nutrient agar (0.7% w/w agar) seeded with target pathogens was spread over the visible colonies of LAB and incubated further at 37°C for 16–20 h.

In agar well method, a lawn of target bacteria over Mueller-Hinton agar (Merck, Germany) with wells of 6 mm bored with sterile borer was used. Cell-free supernatants were obtained by centrifuging 24 h grown MRS broth culture of LAB at 8000 rpm and 4°C for 20 min. The supernatant (50 μ l) of the LAB adjusted to pH 5.5 with 1 M NaOH and HCl and sterilized by passing through filter membranes (0.2 μ m pore size, Minisart, Sartorius Stedim Biotech Co. Ltd., Germany) was added to the well and incubated at 37°C for 16–20 h.

In mix culture study the target bacteria and LAB were grown together in LAPTg broth (1.5% peptone, 1% tryptone, 1% glucose, 1% yeast extract, 0.1% tween-80; pH 6.5) with approximate initial inoculum of LAB (10^5 – 10^6 CFU/mL) and target bacteria (10^4 – 10^5 CFU/mL) and incubated at 37°C for 24–48 h. Growths of and target bacteria were determined by standard plate count using nutrient agar, mannitol salt agar (Himedia, India), xylose lysine deoxycholate agar (Difco, USA) and eosin methylene blue agar (Himedia, India), whereas, the growth of LAB was determined by plate count on MRS agar.

2.3. Probiotics and functional characteristics

The LAB with a broad spectrum of antibacterial activity was selected for further use in biopreservation. Probiotics properties of the selected LAB were examined by following Akbar, Sitara, Ali, Muhammad, and Khan (2014b) and Kaushik et al. (2009).

2.3.1. pH tolerance

Fresh culture (2 μ l) of LAB (10^6 – 10^7 CFU/mL) was inoculated in MRS broths with a pre-adjusted pH of 3.0 with 1 M HCl, 7.0 and 10 with 1 M NaOH separately, and incubated at 37°C for 12–24 h. Normal MRS broth with same bacteria was used as

control. The resistance to pH was determined by plate count method.

2.3.2. Salt and bile salt tolerance

Fresh culture (2 µl) of LAB was introduced into MRS agar plates and broths supplemented with 3% and 6.5% of common salt (NaCl) and 0.3%, 1.0%, and 2.0% of bile salt (Biomark, India) and incubated at 37°C for 4 h. Normal MRS agar and broth were used as control.

2.3.3. Growth at different temperatures

LAB (10^6 – 10^7 CFU/mL) were inoculated to MRS agar and broth and incubated at different temperatures (10, 25, 35 and 44°C) for 24 h to observe visible growth.

2.3.4. Hydrophobicity

Hydrophobicity of isolated LAB was determined by following Akbar et al. (2014b). Fresh LAB culture was centrifuged for 10 min at 8000 rpm and washed with sterile normal saline. Cell suspension was measured for optical density (OD_{600}) (A_0). Toluene (1 mL) was mixed with 3 mL of the suspension and mixed well for 2 min and left standing for 15–20 min. The OD_{600} of the lower aqueous phase (A) was measured. Cell surface hydrophobicity (%H) was calculated using the following equation.

$$\%H = (A_0 - A/A_0) (100)$$

2.4. Partial purification and molecular mass determination of bacteriocin

LAB (5%) was grown in 1,000 mL MRS broth at 37°C for 24 h. Following the incubation, the broth was centrifuged at 8000 rpm for 15 min. The cell-free supernatant was then subjected to 80% ammonium sulfate precipitation (Maldonado-Barragán, Caballero-Guerrero, Martín, Ruiz-Barba, & Rodríguez, 2016). The precipitate was separated by centrifugation at 12,000 rpm for 40 min at 4°C and re-suspended in potassium phosphate buffer (50 mmol/l, pH 7.0) and exhaustively dialyzed overnight through 1000 Da molecular weight-cut-off-dialyzed membrane (Spectra/Por 7 dialysis tubing, 1 K MWCO, 38 mm flat width) against the same buffer. Final solution was filtered through 0.2 µm pore size diameter (Minisart, Sartorius Stedim Biotech Co. Ltd., Germany). Antibacterial activity of partially purified bacteriocin was confirmed by spotting 20 µl on the surface of nutrient agar seeded with target bacteria and incubated at 37°C for 16–24 h.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a 16.5% and 10% gel as described by Schägger and Von (1987). Low molecular-weight peptide markers (BioRad, USA) were used as a standard to compare with the molecular mass of bacteriocin. The gel was stained with Coomassie brilliant blue R-250 (1 g/L) in 50% methanol and 10% acetic acid.

2.5. Protective culture study

The *L. lactis* subsp. *lactis* was applied against *S. aureus* and *Salmonella typhimurium* in pasteurized milk by following the method of Ołdak, Zielińska, Rzepkowska, and Kołożyn-Krajewska (2017), with slight modifications. *Salmonella typhimurium* and *S. aureus* free pasteurized milk was used in this

experiment. The fresh culture of *L. lactis* subsp. *lactis* (5 mL, 10^7 – 10^8 CFU/mL) in sterile normal saline was added to 45 mL of milk, whereas *S. typhimurium* and *S. aureus* (10^6 – 10^7 CFU/mL) were also added to milk samples already inoculated with *L. lactis* subsp. *lactis* and mixed well by vortex and incubated at $35 \pm 2^\circ\text{C}$. The milk samples inoculated with *S. typhimurium*, *S. aureus*, and *L. lactis* subsp. *lactis* separately were used as control using the same incubation conditions. Number of the inoculated bacteria was counted from 0 h to 72 h with the help of standard plate count method using MRS medium for *L. lactis* subsp. *lactis*, Xylose Lysine Deoxycholate agar (XLD) (Difco, USA), for *S. typhimurium* and Mannitol Salt Agar (MSA) (Himedia, India) for *S. aureus*, whereas pH of the milk samples was also recorded each time using pH meter (Hanna, USA). Milk samples inoculated with each bacterium and without bacteria were used as a control in the study.

2.6. Biopreservation of poultry meat by bacteriocin

A challenge study for the biopreservation of fresh poultry meat was conducted using *S. typhimurium* and *S. aureus* as target bacteria by following the method of Akbar and Anal (2014a) with slight modifications. Meat pieces with a surface area of 4 cm², approximately equal to 10 g were contaminated with target bacteria (10^5 – 10^6 CFU/g). Partially purified bacteriocin (10 µg/g of meat) was spread equally on the surface of the contaminated meat pieces with the help of hand operated sterile spray bottles and kept at $6 \pm 2^\circ\text{C}$ for four days. The number of target bacteria was counted with the help of standard plate count method using XLD and MSA. Bacteriocin free samples incubated at the same condition were used as control.

2.7. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey test to determine significant group differences ($p < 0.05$) between samples by using SPSS statistical software package (SPSS, version 23.0, USA).

3. Results and discussion

3.1. Isolation and antagonistic activity of lactic acid bacteria

Six different species were found in all 25 fermented milk samples analyzed for the presence of potential bacteriocinogenic LAB (Table S1). Based on the morphological and microscopic characteristics bacterial isolates were found to be bacilli (rods), and predominantly cocci (round) in short and long chain. The bacteria were biochemically identified with the help of API kits, and the antimicrobial spectrum against foodborne pathogens was determined. On the bases of prominent antimicrobial spectrum *L. lactis* subsp. *lactis* (Table S1) was further analyzed for its probiotic characteristic, bacteriocin extraction and for biopreservation. *Lactococcus lactis* subsp. *lactis* were found active against wide range of foodborne pathogens, used as target bacteria in this study. Rodriguez et al. (2012) reported that only 52 out of all 169 LAB isolates from infant feces showed inhibition zone higher than 10 mm against *Escherichia coli* and *Listeria innocua*. Malini and Savitha (2012) reported 135 isolates of LAB from food sources including cheese, paneer, and

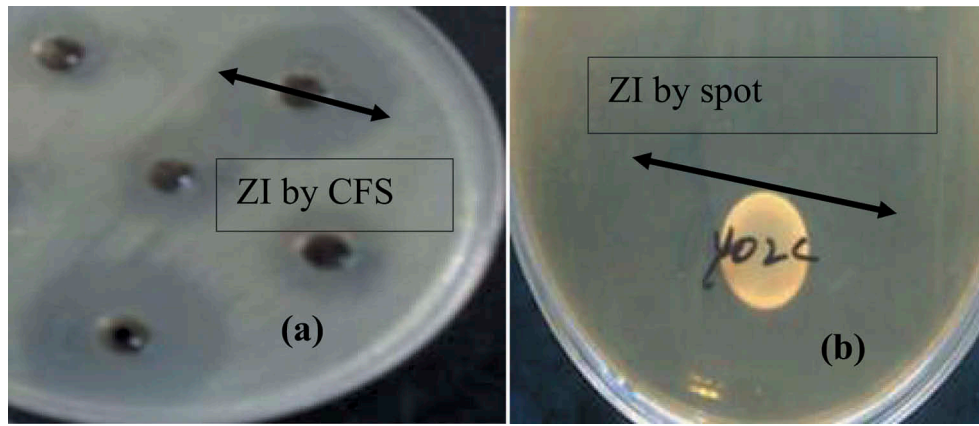


Figure 1. Zone of inhibition of LAB and its metabolites against target bacteria (a) Zone of inhibition (ZI) of CFS (cell-free supernatant) of *L. lactis* by agar well diffusion and (b) Zone of inhibition (ZI) of *L. lactis* by spot on lawn method.

Figura 1. Zona de inhibición de LAB y sus metabolitos contra bacterias diana (a) Zona de inhibición (ZI) de CFS (sobrenadante libre de células) de *L. lactis* por difusión en agar y (b) Zona de inhibición (ZI) de *L. lactis* por el método de mancha en el césped "spot on lawn method."

sausages, belonged to the groups *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Bifidobacterium*, and reported that only 75% of the isolates showed antibacterial activity.

The highest antibacterial activity of *L. lactis* subsp. *lactis* was found against *Listeria monocytogenes* (27.3 ± 1.4 mm) and *Staphylococcus aureus* (21.2 ± 1.2 mm). The antimicrobial activity against *Salmonella typhimurium* presented an inhibition zone of 11.2 ± 1.72 mm, whereas for *Escherichia coli* 13.4 ± 1.15 mm (Figure 1.). The *L. lactis* subsp. *lactis* were found effective against the target bacteria in mix culture study in LAPTg broth and all the target bacterial growth were significantly ($p < 0.05$) reduced to undetectable (zero CFU/mL) from initial inocula 10^5 – 10^6 CFU/mL after 24–48 h incubation confirmed by standard plate count. Guessas, Hadadji, Saidi, and Kihal (2007) reported the antagonistic effect of *L. lactis* subsp. *lactis* in agar plates and liquid milk. In this study *L. lactis* subsp. *lactis* isolated from fermented milk products showed antimicrobial potential against different tested pathogens. LAB previously isolated from fermented Indian products and cocoa fermentation were found effective against wide range of pathogenic bacteria; moreover, the antimicrobial effects of LAB were similar to the *L. lactis* subsp. *lactis* isolated in the current study (Angmo et al., 2016; Santos et al., 2016). Moreover, Ojha, Kerry, Alvarez, Walsh, and Tiwari (2016) reported that cell-free extracts of *Lactobacillus sakei* obtained after fermentation of ultrasound treated samples showed remarkable antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* at lower concentrations compared to control.

3.2. Probiotics characteristic

Probiotic characteristics of *L. lactis* subsp. *lactis* were determined by exposing to different ranges of pH and temperature, different concentrations of salt and bile salt and its percentage of hydrophobicity (Table 1). *L. lactis* subsp. *lactis* growth in acidic (pH 3) MRS broth decreased by 70% after 12 h incubation, whereas normal growth was observed in neutral (pH 7), and no media growth was observed in alkaline (pH 10) conditions. The bacterium was found tolerant to 3% salt (NaCl) concentration, whereas no growth was observed in 6.5% salt. *L. lactis* subsp. *lactis* were able to grow at

Table 1. Probiotic and functional characteristics of *L. lactis* subsp. *lactis* isolated from fermented milk.

Tabla 1. Características probióticas y funcionales de *L. lactis* subsp. *lactis* aislada de leche fermentada.

No	Characteristics	Results
1	Growth in pH	
	3	±
	7	+ve
2	Growth in bile salt	
	0.3%	+ve
	1.0%	±
3	Growth in salt (NaCl)	
	3.0%	+ve
	6.5%	-ve
4	Growth on temperature (°C)	
	10	+ve
	25	+ve
	35	+ve
5	% of Hydrophobicity	61

+ ve = Growth of bacteria, -ve = No growth of bacteria, ± = Reduction in number of bacteria (initially inoculated).

+ ve = Crecimiento de bacterias, -ve = Sin crecimiento de bacterias, ± = Reducción en el número de bacterias (inicialmente inoculadas).

temperatures 10, 25 and 35°C and no growth was observed at 44°C. The growth was found persistent in bile salt concentration 0.3%, while decrease in initial bacterial count was observed with increasing concentration of bile salt in the media. The percentage of hydrophobicity for *L. lactis* subsp. *lactis* was 61%. Pan and Zhang (2008) reported good growth of *L. lactis* subsp. *lactis* in 0.1% bile salt and decrease in growth with the increasing bile salt concentration. They also reported the growth stability of *L. lactis* subsp. *lactis* at low pH (2.5 and 4.5) and the growth was maintained above 10^8 CFU/mL for 6 h. Resistance of LAB to bile salt concentration of 0.3% is considered as a good probiotics characteristic (Rodriguez et al., 2012).

3.3. Protective culture study

The putative probiotic bacteria *L. lactis* subsp. *lactis* used against *S. typhimurium* and *S. aureus* in milk as a protective culture were found active against both of the target bacteria. It was observed that the pathogenic bacterial growth was

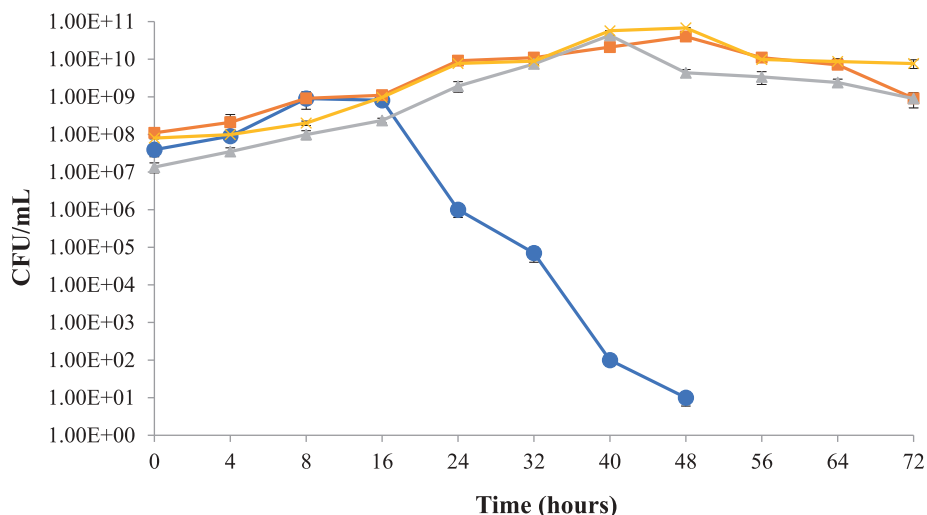


Figure 2. Protective culture study in milk using *S. aureus* and *L. lactis* subsp. *lactis* at $35 \pm 2^\circ\text{C}$ storage, *Staphylococcus aureus* (●), *Lactococcus lactis* subsp. *lactis* (■) *Lactococcus lactis* subsp. *lactis* control (x), *Staphylococcus aureus* control (▲).

Figura 2. Estudio de cultivo protegido en leche utilizando *S. aureus* y *L. lactis* subsp. *lactis* almacenado a $35 \pm 2^\circ\text{C}$, *Staphylococcus aureus* (●), *Lactococcus lactis* subsp. *lactis* (■) *Lactococcus lactis* subsp. *lactis* control (x), *Staphylococcus aureus* control (▲).

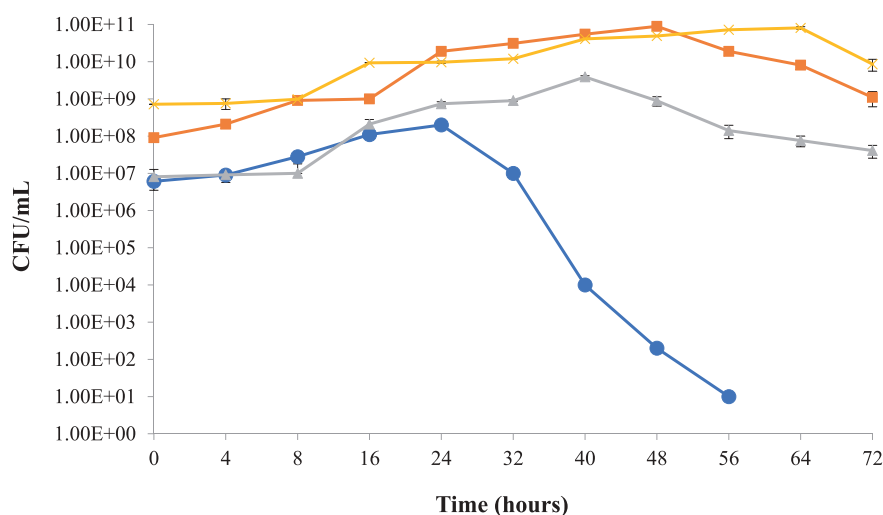


Figure 3. Protective culture study in milk using *Salmonella typhimurium* and *L. lactis* subsp. *lactis* at $35 \pm 2^\circ\text{C}$ storage, *Salmonella typhimurium* (●), *Lactococcus lactis* subsp. *lactis* (■) *Lactococcus lactis* subsp. *lactis* control (x), *Salmonella typhimurium* control (▲).

Figura 3. Estudio de cultivo protegido en leche utilizando *Salmonella typhimurium* y *L. lactis* subsp. *lactis* almacenado a $35 \pm 2^\circ\text{C}$, *Salmonella typhimurium* (●), *Lactococcus lactis* subsp. *lactis* (■) *Lactococcus lactis* subsp. *lactis* control (x), *Salmonella typhimurium* control (▲).

significantly ($p < 0.05$) reduced with the passage of time (Figures 2 & 3). Target bacterial growth was initially not hindered by the growth of *L. lactis* subsp. *lactis* in a mix culture but subsequent reduction was noted after 24 h of incubation. Such condition might be due to the adjustment of *L. lactis* subsp. *lactis* to the environment initially and possibly due to the production of antibacterial compounds after the limitation of nutrients in the milk. The pH of test milk samples also dropped to 5.2 from initial pH 6.8, after 24 h incubation time. Akbar and Anal (2014a) reported the tenfold reduction of *S. aureus* in a similar study conducted for the preservation of ready to eat poultry meat.

3.4. Biopreservation of poultry meat

The molecular mass of partially purified bacteriocin from *L. lactis* and standard low molecular weight protein marker

is presented in Figure 4. The diffused bands were observed for partially purified bacteriocin in the range of 14,437–6512 Dalton. This might be due to the crude nature of metabolites and other interfering molecules. The partially purified bacteriocin was found effective against test foodborne pathogens (*S. typhimurium* and *S. aureus*) used in the study. The partially purified bacteriocin was found active against the target bacteria in poultry meat, and the target bacterial count 10^5 – 10^6 CFU/g was significantly ($p < 0.05$) reduced to 10^1 CFU/g in case of *S. aureus* and 10^2 CFU/g in case of *S. typhimurium* after three days of incubation at low temperature ($6 \pm 2^\circ\text{C}$) (Figure 5).

The use of LAB and its antimicrobial metabolites such as organic acid, hydrogen peroxide, and bacteriocin, is a promising ongoing development in food preservation with environmental friendly measures (Jofré et al., 2008). Todorov et al. (2017) reported that bacteriocin isolated

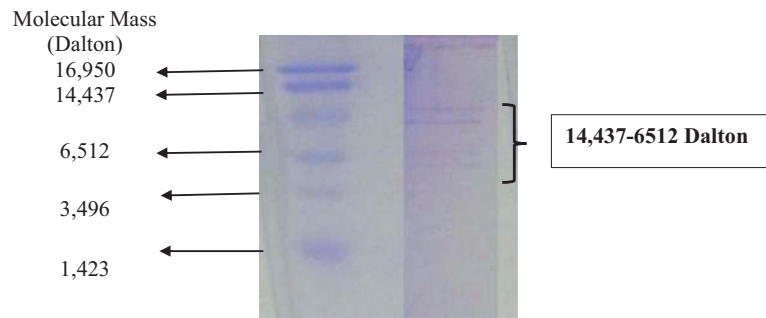


Figure 4. Tricine SDS-PAGE for molecular mass analysis of partially purified bacteriocin from *Lactococcus lactis* subsp. *Lactis*. Diffused bands in the range of 14437–6512 Daltons.

Figura 4. SDS-PAGE de tricina para el análisis de la masa molecular de la bacteriocina parcialmente purificada de *Lactococcus lactis* subsp. *lactis*. Bandas difusas en el rango de 14437–6512 Daltons.

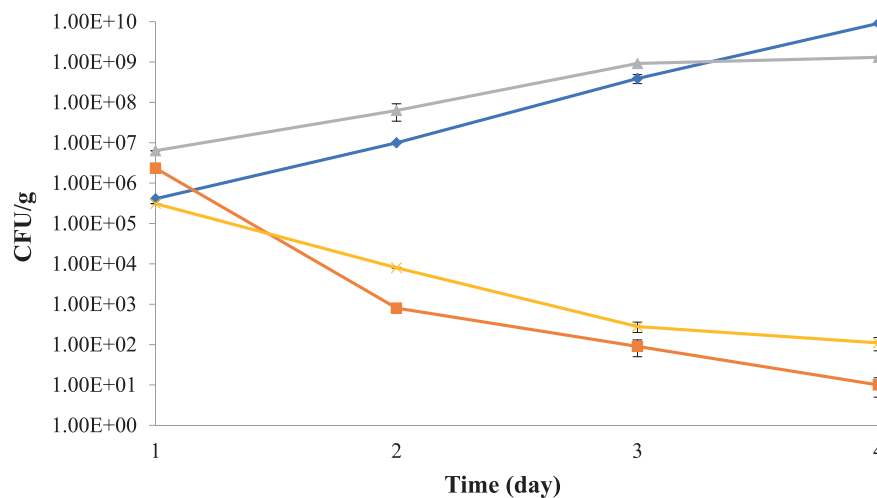


Figure 5. Growth of *Staphylococcus aureus* and *Salmonella typhimurium* in fresh poultry meat in the presence of crude bacteriocin at $6 \pm 2^\circ\text{C}$ storage, *Salmonella typhimurium* (×), *Staphylococcus aureus* (■) *Salmonella typhimurium* control (◆), *Staphylococcus aureus* control (▲).

Figura 5. Crecimiento de *Staphylococcus aureus* y *Salmonella typhimurium* en carne de ave fresca en presencia de bacteriocina en bruto almacenada a $6 \pm 2^\circ\text{C}$, *Salmonella typhimurium* (×), *Staphylococcus aureus* (■) *Salmonella typhimurium* control (◆), *Staphylococcus aureus* control (▲).

from LAB was found effective against *L. monocytogenes* and various other pathogens indicating the applications of bacteriocinogenic LAB in biopreservation of food products.

Antibacterial activity of meat-borne LAB has been reported in biopreservation of cooked meat products (Vermeiren, Devlieghere, & Debevere, 2004). Twomey et al. (2000) reported broad antibacterial activity of *L. lactis*. Jofre et al. (2008) reported the inactivation of *Salmonella* and *L. monocytogenes* by bacteriocin in combination with high hydrostatic pressure in ready-to-eat meat products. The antibacterial activity of the *L. lactis* subsp. *lactis* against other bacteria is due to its ability to produce a variety of antibacterial compounds including organic acids, hydrogen peroxides and bacteriocin (Rodgers, 2003).

4. Conclusion

It was concluded that LAB with antimicrobial activity has promising capabilities for its use against spoilage and pathogenic microorganisms in food. *L. lactis* subsp. *lactis* has the potential to control the number of unwanted bacteria in poultry meat and milk products. The use of *L. lactis* subsp. *lactis* as a starter culture or protective culture and its

bacteriocin as antimicrobial agents in meat and milk products can prevent the growth of foodborne pathogens in the respective food products. However, further purification and identification of antimicrobial metabolites of probiotics, isolated from traditionally fermented foods can be a new source for novel and natural preservatives. Isolation of novel bacteriocin and other antimicrobials from environmentally friendly sources for biopreservation purpose is the demand for food industries and society for sustainability.

Disclosure statement

No potential conflict of interest was reported by the authors.

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