Isolation of antimicrobial lactic acid bacteria with potential use as a protective culture for 'Queso Fresco'

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Abstract

Lactic acid bacteria (LAB) isolated from various sources are used in the food industry for different purposes depending on their characteristics; being its antimicrobial capacity one of the most important. The objective of this work was to determine the antagonism of LAB's isolated from milk and its derivatives against *Salmonella*, *Staphylococcus* and *Listeria*, contaminants of 'queso fresco' produced in the south of the state of Chiapas, Mexico, as a strategy for the development of protective crops for this artisan cheese. A total of 54 samples of dairy products (whey, milk and cheeses) from the Istmo-Costa region were analyzed. 52 strains of LAB were isolated, which were analyzed to investigate their antibacterial capacity by the methods of the drop on the surface and diffusion in agar. From the first method, 36 strains evaluated showed signs of being antagonistic to pathogens: nine against *Staphylococcus aureus*, seven against *Salmonella* sp. and five against *Listeria monocytogenes*. In the agar diffusion test, 31 strains evaluated showed antagonistic capacity: eight against *S. aureus*, 12 against *Salmonella* sp. and 11 against *L. monocytogenes*. These results encourage one to think that LAB's isolated from dairy products may function as protective cultures from pathogens that contaminate 'queso fresco'. Additional testing is required to validate this hypothesis.

Keywords:
Dairy products
Inhibition
Listeria monocytogenes
Salmonella sp.
Staphylococcus aureus

Aislamiento de bacterias lácticas antimicrobianas con potencial uso como cultivo protector para Queso Fresco

Resumen

Las bacterias ácido lácticas (BAL) aisladas de diversas fuentes son utilizadas en la industria de los alimentos con distintos propósitos, dependiendo de sus características; siendo su capacidad antimicrobiana una de las más importantes. El objetivo de este trabajo fue determinar el antagonismo antimicrobiano de las BAL aisladas de leche y sus derivados contra *Salmonella*, *Staphylococcus* y *Listeria*, contaminantes del queso fresco producido en el sur del estado de Chiapas, México, como estrategia para el desarrollo de cultivos protectores para este queso artesanal. Se analizaron un total de 54 muestras de productos lácteos (suero, leche y quesos) de la región Istmo-Costa. Se aislaron 52 cepas de BAL, las cuales fueron analizadas para investigar su capacidad antibacteriana por los métodos de la gota sobre la superficie y difusión en agar. Del primer método, 36 cepas evaluadas demostraron antagonismo contra los patógenos: nueve contra *Staphylococcus aureus*, siete contra *Salmonella* sp. y cinco contra *Listeria monocytogenes*. En la prueba de difusión en agar, 31 cepas evaluadas mostraron capacidad antagónica: ocho contra *S. aureus*, 12 contra *Salmonella* sp. y 11 contra *L. monocytogenes*. Estos resultados hacen pensar que las BAL aisladas de productos lácteos pueden funcionar como cultivos protectores de patógenos que contaminan el queso fresco. Pruebas adicionales son requeridas para validar tal hipótesis.

Palabras clave:
Productos lácteos
Inhibición
Listeria monocytogenes
Salmonella sp.
Staphylococcus aureus

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1. Introduction

As is the case in many regions of the world, artisan cheeses are widely consumed food products in Mexico. Of these, the preference for 'queso fresco' stands out, which represent one of the most important types, with a production of 89 557 tons per year (SIAP, 2020). These cheeses are made almost exclusively from raw milk (unpasteurized), which freshly milked has a low bacterial presence, however, in subsequent processes, there is the possibility of contamination from various sources (Willis et al., 2022). The pasteurization of milk is the first option to reduce the microbiological load and it is also a regulatory requirement (NOM-243-SSA1-2010), however, it is still not used among small and medium producers, since during pasteurization they are also destroyed desirable bacteria, responsible for the specific sensory characteristics, which detracts from the acceptability of the products by consumers (González-Córdova et al., 2016).

Biopreservation, on the other hand, is a method that offers various conditions to extend the shelf life and increase the safety of fresh cheeses, through the implementation of either native or exogenous microbiota (protective cultures) and/or its microbial products (Todorov, 2019). Different studies have used this procedure, mainly using bacteria belonging to the natural microbiota of various products, such as lactic acid bacteria (LAB) isolated from dairy, meat, fish and vegetable products (Aragon-Alegro et al., 2021; Martín et al., 2022). This procedure makes use of the antibacterial properties, attributed to the final products of LAB metabolism such as lactic acid, acetic acid, diacetaldehyde, reuterin and bacteriocins, among the main ones (Londoño et al., 2015). In addition, it has been shown that this preservation option offers better results, when LABs obtained from similar environments are applied to where they are to be applied (Punia-Bangar et al., 2022). Fuentes et al. (2017) isolated 32 strains of BAL from 'doble crema' cheese and 'quesillo' (Oaxaca cheese), 13 strains showed antagonistic capacity against *Listeria monocytogenes* and against *Salmonella typhimurium*. Also, Peralta (2014) obtained 75 strains of BAL, of which 10 strains, presented antagonistic capacity against *L. monocytogenes* and *Escherichia coli*.

In the Istmo-Costa region, in the state of Chiapas, in southern Mexico, various artisan cheeses are produced with unpasteurized milk, which can be potentially contaminated with human pathogens as occurs in cheeses from other regions of the country (Guzmán -Hernández et al., 2016a; Ibarra-Sánchez et al., 2017). To date, the information on undesirable or pathogenic microorganisms that can be found in Chiapas cheeses is scarce but given the similarity in the characteristics of the cheeses and the production procedures, it is highly probable to find bacteria such as *Salmonella, Staphylococcus* or *Listeria*, as reported in 'queso fresco' made in the Mexican state of Tabasco (Guzmán-Hernández et al., 2016a; 2016b).

This work had two main purposes, to verify the presence of pathogens in 'queso fresco' made and marketed in the coastal strip of the state of Chiapas; as well as isolate lactic acid bacteria from milk and dairy derivatives that show antimicrobial activity specifically against *Salmonella, Staphylococcus* and *Listeria*; bacteria considered contaminants in fresh cheeses; as a strategy for the development of protective cultures.

2. Materials and Methods

2.1. Isolation of lactic acid bacteria

As raw material for the isolation of lactic acid bacteria (LAB), six types of samples were collected in triplicate: 1) freshly milked milk, 2) freshly whey obtained from the cheese factory, 3) 'doble crema' cheese, 4) quesillo (Oaxaca cheese), 5) 'queso fresco' and 6) Cotija cheese; all samples from the Istmo-Costa region. For the milk and whey samples, 500 mL were collected in sterile plastic containers, and, for the cheese samples, 500 g of cheese were purchased and placed in sterilized hermetic bags. This procedure was repeated three more times for the cheese samples, and each sample was treated as independent, making a total of 54 samples that were at all times transferred on ice to the laboratory for immediate analysis.

From each cheese samples, 10 g of each were weighed and placed in a sterile blender containing 90 mL of sterile peptone water (SPW). After low-speed liquefaction, the suspensions were placed in an Erlenmeyer flask. For the liquids (milk and whey), 10 mL of each were measured and they were added to 90 mL of SPW to proceed to homogenize the solution.

Subsequently, 100 µL of each suspension were taken and seeded in triplicate by casting into Petri dishes containing de Man Rogosa and Sharpe (MRS) agar. The plates were incubated at 37 °C under anaerobic conditions in anaerobic chamber. With the colonies grown, the isolation process was started by taking with a handle the colonies that were morphologically different in the edges (circular or irregular, wavy or smooth), the surface (convex or flat) and the color (white, yellowish reddish, opaque, creamy and shiny). For consecutive reseeding, the selective medium from which the strain was obtained was used and they were placed under anaerobic conditions for 24-48 h until a single colony morphology per plate was observed. Subsequently, each of the strains underwent the catalase and peroxidase tests. The strains that turned out to be catalase and peroxidase negative were smeared, fixed with heat and stained by the Gram method to verify the microscopic morphology and staining with the help of an Axiolab® optical microscope with an image analyzer, selecting those that resulted Gram positive and presented bacilli form (Vázquez-Velázquez et al., 2018).

2.2. Isolation of pathogenic bacteria

As raw material for the isolation of pathogenic bacteria, four samples of 'queso fresco' were collected in triplicate from four cheese factories in the Istmo-Costa region. All samples (repetitions) were treated independently, obtaining a total of 12 samples, which were later transferred on ice to the laboratory for immediate analysis.

For the isolation of *L. monocytogenes*, 25 g of 'queso fresco' were weighed in a sterile bag, the sample was macerated and 100 mL of enrichment broth with a *Listeria* supplement were added. The samples were homogenized, placed in bottles with a hermetic closing lid, and incubated at 35 °C for 48 h. Subsequently, 0.5 mL of the samples were seeded in Oxford selective medium, the seeded plates were incubated for 48 h at 37 °C. Colonies with characteristic *Listeria* morphology underwent Gram staining, mobility test, catalase and hemolysis (Guzmán-Hernández et al., 2016a).

For the isolation of *Salmonella* spp., the method of Guzmán-Hernández et al. (2016a) with slight modifications was followed. 25 g of 'queso fresco' previously macerated under sterile conditions were added to 225 mL of peptone water for homogenization. The samples were transferred to bottles with a hermetic closing cap and incubated at 37 °C for 24 h. After incubation, 1 mL of the sample was transferred to 10 mL of selenite-cysteine broth, and 1 mL to 10 mL of tetrathionate broth. Both medias were incubated at 37 °C. After 24 h, 10 µL of each medium was taken and spread onto the Salmonella-Shigella agar, Xylose Lysine Deoxycholate (XLD) agar, MacConkey agar, and incubated at 37 °C for 24 h. Three to five colonies with characteristic of *Salmonella* morphology were selected from each medium for their biochemical confirmation with the Triple Sugar Iron (TSI), Lysine Iron agar (LIA) and urease production tests.

For *Staphylococcus aureus* isolation, 10 g of 'queso fresco' previously macerated in sterile conditions, were added to 90 mL of phosphate buffer and homogenized. Decimal serial dilutions were made from the homogenate. 0.1 mL of each dilution were taken and spread in Petri dishes containing Baird-Parker agar (BPA) supplemented with potassium tellurite and egg yolk emulsion. The plates were incubated at 37 °C for 48 h. Those black colonies with transparent halo were selected, to later perform the Gram staining tests, coagulase, thermonuclease and mannitol for the identification of *S. aureus* (Guzmán-Hernández et al. 2016a).

2.3. Antagonistic capacity of LABs

For the drop-on-surface method, the LAB isolates were transferred to tubes containing 5 mL of MRS broth and incubated at 37 ºC overnight, then they were spotted (20 µL) on MRS agar plates under anaerobic conditions for 12 h at 37 ºC. The pathogenic bacteria (*Salmonella* sp., *Listeria monocytogenes* and *Staphylococcus aureus*) were individually incubated in tubes with 5 mL of brain-heart infusion (BHI) broth (Oxoid) at 37 ºC for 12 h under anaerobic conditions. Then, 100 µL of the suspension was transferred to 10 mL of fresh BHI broth. The mixture was completed with 0.75% bacteriological agar and placed in a water bath at 45 ºC. Each mixture containing cells of the pathogenic bacteria was poured (overlaid) onto the LAB-cultured plates. After completing semi-solidification of the upper layer, the plates were incubated for 24 h at 37 °C under anaerobic conditions. The antagonistic activity of LABs against pathogenic bacteria was confirmed by the formation of inhibition halos; halos were measured, and LABs were chosen for the modified plate diffusion method (Lima et al., 2007).

For the plate diffusion method, LAB´s strains were grown in MRS broth at 37 ºC until reaching the stationary phase (12-16 h). Subsequently, the media were centrifuged at 6 860 *xg* for 10 min. CFS were used in the antimicrobial assay that was performed by the plate diffusion method. 50 µL of CFS was placed in a 6 mm diameter well perforated on BHI agar, which was previously inoculated with 10 µL of a culture of the pathogenic strains. For this, *L. monocytogenes*, *Salmonella* sp. or *Staphylococcus aureus* were grown in BHI broth up to an optical density of 0.102 read at 600 nm, which corresponds to an approximate 10^6 CFU.mL^{-1}. The plates were incubated at 37 ºC for 24 h (Lima et al., 2007), the growth of the LAB was monitored and the diameter of the zone of inhibition (mm) around the wells (including the well), it was measured with a Vernier caliper (three times).

2.4. Data analysis

The data obtained from the inhibition tests were subjected to analysis of variance and subsequent multiple comparison of ranges by Fischer's least significant difference (LSD) procedure ($\alpha = 0.05$). This analysis was performed using Statgraphics Centurion XV v. 15.2.06 software.

3. Results and Discussion

3.1. Lactic acid bacteria isolated

One hundred and five bacterial isolates were obtained from freshly milked milk, fresh whey and cotija, 'fresco', 'quesillo' and 'doble crema' cheeses. Of these isolates, only 52 presented characteristic LAB morphologies. 32 had a point shape, 12 circular and 8 irregulars; all showed round edge and flat surface. All strains being catalase and peroxidase negative and Gram positive. The isolates were coded with a letter that signifies the type of cheese or milk, sample and a sequential number (Table 1).

The total number of strains obtained in our study is lower than that reported by Luiz et al. (2017) with a similar product (Minas cheese) from Brazil. This difference may be due to the number of samples (84) and the seven ripening times (cheese) that were considered for that study. Only fresh milk, whey and cheeses were analyzed in our study.

3.2. Pathogens isolated

From 'queso fresco' from four different sources (12 samples in total), 20 isolates were obtained in the selection culture media. Of these isolates, 10 strains were positive for the pathogenic microorganisms of interest. Three positive isolates for *Staphylococcus aureus*, four with characteristics of *Salmonella* spp. and three isolated from *Listeria monocytogenes* (Table 2). The pathogenic strain selected (of each type) for the confrontations against LAB, was the one that exhibit the best response to growth and came from the sample with the highest prevalence of pathogens.

Table 1. Morphological characteristics of lactic acid bacteria isolated from dairy products from the coastal region of Chiapas, Mexico.

Strain	Elevation	Form	Surface	Strain	Elevation	Form	Surface
CM1.C1	Round	Punctiform	Flat	CM3.C1	Round	Punctiform	Flat
CM1.C2	Round	Circular	Flat	CM3.C2	Round	Punctiform	Flat
CM1.C3	Round	Punctiform	Flat	CM3.C3	Round	Punctiform	Flat
DCM1.C1	Round	Punctiform	Flat	DCM3.C1	Round	Punctiform	Flat
DCM1.C2	Round	Punctiform	Flat	DCM3.C2	Round	Punctiform	Flat
DCM1.C3	Round	Punctiform	Flat	DMC3.C3	Round	Punctiform	Flat
FM1.C1	Round	Punctiform	Raised	FM3.C1.1	Round	Punctiform	Flat
FM1.C3	Round	Circular	Flat	FM3.C1.2	Round	Punctiform	Flat
FM1.C4	Round	Irregular	Flat	FM3.C2	Round	Punctiform	Flat
FM1.C5	Round	Irregular	Flat	FM3.C3	Round	Punctiform	Raised
FM1.C6	Round	Punctiform	Flat	CM4.C1	Round	Circular	Flat
FM1.C10	Round	Circular	Flat	CM4.C3	Round	Punctiform	Flat
QM1.C3	Round	Circular	Flat	QM3.C1	Round	Punctiform	Flat
QM1.C5	Round	Punctiform	Flat	QM3.C2	Round	Punctiform	Flat
CM2.C1	Round	Irregular	Flat	QM3.C3	Round	Punctiform	Flat
CM2.C3	Round	Punctiform	Flat	CM4.C2	Round	Punctiform	Flat
CM2.C5	Round	Punctiform	Flat	DCM4.C1	Round	Circular	Flat
DCM2.C1	Round	Punctiform	Flat	DCM4.C2	Round	Irregular	Raised
DCM2.C2	Round	Punctiform	Flat	DCM4.C3	Round	Irregular	Flat
DCM2.C3	Round	Punctiform	Flat	FM4.C1	Round	Irregular	Flat
FM2.C2	Round	Punctiform	Flat	FM4.C2	Round	Circular	Flat
FM2.C3	Round	Punctiform	Flat	QM4.C1	Round	Circular	Flat
FM2.C4	Round	Circular	Flat	QM4.C2	Round	Punctiform	Flat
QM2.C1	Round	Irregular	Flat	QM4.C3	Round	Circular	Flat
QM2.C2	Round	Circular	Flat	LM3.C3	Round	Punctiform	Flat
QM2.C3	Round	Punctiform	Flat	SM1.C2	Round	Irregular	Flat

In the strain code, C: Cotija cheese; F: 'queso fresco'; DC: 'doble crema' cheese**;** Q: 'quesillo'; L: milk; S: whey; M: sample

Table 2. Prevalence of pathogenic bacteria in 'queso fresco' samples collected in the coastal region of Chiapas, Mexico.

Cheese	Sample	*Staphylococcus aureus*	*Salmonella* sp.	*Listeria monocytogenes*
Q1	1	+	+	+
	2	-	+	+
	3	-	+	-
Q2	1	+	-	-
	2	+	+	+
	3	+	+	+
Q3	1	-	+	+
	2	-	+	+
	3	-	-	+
Q4	1	+	+	-
	2	+	-	-
	3	-	+	-

+: presence, -: absence

All the cheeses analyzed (Q1-Q4) in this study were positive for the presence of *Salmonella* spp., while 75% of the samples were positive for both *S. aureus* and *L. monocytogenes*. González-Montiel and Franco-Fernández (2015) report that, from 12 cheese samples of the Cañada Oaxaqueña, all tested positive for *S. aureus* and *Salmonella* spp., the results being very similar to ours. Guzmán-Hernández et al. (2016a), report that of 52 cheese samples from the Gulf of Mexico, 19 presented *S. aureus*, one cheese was positive for *L. monocytogenes* and two for *Salmonella* spp. In another study carried out with 52 samples of cheese from Tabasco (Mexico), only two samples were positive for *Salmonella* spp. (Guzmán-Hernández et al., 2016b). Our results were discordant in the number of strains obtained of *L. monocytogenes* and *S. aureus* with these authors, this may be since these samples are from different geographical region (different environmental conditions and manufacturing practices), as well as the types of cheese analyzed, but they coincide in the presence of pathogens. The mere presence of pathogens in cheeses already represents a serious problem for the health of consumers and may be due to the lack of hygienic-sanitary conditions that usually appear from the beginning of the process in the handling of milking to the preservation of the final product (cheese) at the points of sale (Guzmán-Hernández et al., 2016b).

3.3. Antagonistic capacity of LABs against pathogenic bacteria

From 52 strains of LAB, antagonism tests were carried out by direct confrontation between the LAB and the pathogen by the method of the drop on the surface. From this test, 36 strains (69.23%) with antagonistic capacity were obtained; of which 24 had activity against a single genus of pathogen (14 against *S. aureus*, nine against *Salmonella* sp. and one against *L. monocytogenes*). 11 LABs showed activity against two genera of pathogens (six against *S. aureus* and *Salmonella* sp., one against *S. aureus* and *L. monocytogenes* and four against *Salmonella* sp. and *L. monocytogenes*). Among the

BAL strains, the FM4.C1.2 strain stands out, which exhibited antagonism against the three pathogens tested in this study, through the absence of pathogen growth (Table 3).

Rivera de la Cruz et al. (2017) obtained 11 strains that formed inhibition halos against *Salmonella enterica* var. *Typhimurium*, which agrees with our results. Similarly, in another work by Ferrari et al. (2016) reported three different strains of LAB with the ability to stop the growth (inhibition halos) of *Salmonella Typhi*, *S. aureus* and *L. monocytogenes*, respectively.

Table 3. Lactic acid bacteria strains isolated from dairy products with antibacterial capacity.

LAB strain	Inhibiting bacteria	LAB strain	Inhibiting bacteria
CM1.C1	*Staphylococcus aureus*	CM4.C2	*Salmonella* sp.
CM1.C2.1	*Staphylococcus aureus*	DCM2.C3	*Salmonella* sp.
CM3.C2	*Staphylococcus aureus*	DCM3.C1.2	*Salmonella* sp.
CM4.C2	*Staphylococcus aureus*	DCM3.C2.1	*Salmonella* sp.
DCM1.C1	*Staphylococcus aureus*	FM1.C1	*Salmonella* sp.
DCM1.C5.1	*Staphylococcus aureus*	FM1.C4	*Salmonella* sp.
DCM2.C1	*Staphylococcus aureus*	FM1.C10.2	*Salmonella* sp.
DCM2.C3	*Staphylococcus aureus*	FM2.C3	*Salmonella* sp.
DCM3.C1.2	*Staphylococcus aureus*	FM3.C1.1	*Salmonella* sp.
DCM3.C3	*Staphylococcus aureus*	FM4.C1.1	*Salmonella* sp.
DCM4.C1.1	*Staphylococcus aureus*	FM4.C1.2	*Salmonella* sp.
FM1.C5	*Staphylococcus aureus*	QM1.C3	*Salmonella* sp.
FM2.C3	*Staphylococcus aureus*	QM1.C5	*Salmonella* sp.
FM3.C1.2	*Staphylococcus aureus*	QM2.C2	*Salmonella* sp.
FM4.C1.2	*Staphylococcus aureus*	QM4.C1	*Salmonella* sp.
QM1.C5	*Staphylococcus aureus*	QM4.C3	*Salmonella* sp.
QM2.C1	*Staphylococcus aureus*	CM1.C1	*Listeria monocytogenes*
QM2.C4	*Staphylococcus aureus*	CM3.C3.2	*Listeria monocytogenes*
QM2.C5	*Staphylococcus aureus*	DCM3.C2.1	*Listeria monocytogenes*
QM3.C2.1	*Staphylococcus aureus*	DCM4.C2.1	*Listeria monocytogenes*
QM3.C2.2	*Staphylococcus aureus*	FM1.C1	*Listeria monocytogenes*
CM1.C.2.1	*Salmonella* sp.	FM3.C1.1	*Listeria monocytogenes*
CM3.C2	*Salmonella* sp.	FM4.C1.2	*Listeria monocytogenes*

In the strain code, C: Cotija cheese; F: 'queso fresco'; DC: 'doble crema' cheese**;** Q: 'quesillo'; L: milk; S: whey; M: sample

The CFS of the same 52 strains of BAL were challenged against pathogens by the plate diffusion method. In this test, 21 CFS (40.28%) with antagonistic capacity were obtained. 13 CFS showed activity against a single genus of pathogen (seven against *Salmonella* sp., four against *L. monocytogenes* and two against *S. aureus*). Six CFS showed activity against two genera of pathogens (three against *S. aureus* and *L. monocytogenes*, two against *Salmonella* sp. and *L. monocytogenes* and one against *S. aureus* and *Salmonella* sp.).

The CFS of the strains CM3.C2 and FM4.C1.2, inhibited the three pathogens tested in this study, with inhibition halos ranging from 9 mm to 25.5 mm. The CFS of the strains CM1.C1 and QM1.C5 also stood out, which inhibited two of the three pathogens with the highest inhibition halos. The strain CM1.C1 inhibited to *L. monocytogenes* (8 mm diameter) and *S. aureus* (25 mm diameter); while the QM1.C5 strain inhibited to *Salmonella* sp. (25 mm diameter) and *L. monocytogenes* with 14 mm of diameter (Table 4).

The amount of CFS with antibacterial capacity obtained in our work was greater than that obtained by Arrioja-Bretón et al. (2020), who report that 12 CFS were antagonistic to *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *Salmonella Typhimurium* and *L. monocytogenes*. However, the results regarding the diameter of inhibition were similar, since the authors report values of 28.35, 20.45, 27.71 and 25.83 mm, respectively. In another work reported by De Almeida et al. (2015) obtained six CFS with antagonistic capacity against *E. coli*, *S. aureus*, *Salmonella* spp. and *L. monocytogenes*. Asurmendi et al. (2015) obtained 21 CFS antagonistic to *L. monocytogenes*, the strain B87 being notable with a 24.5 mm inhibition diameter. These results demonstrate that the CFS of the isolates in our study are efficient both in number and in the size of the inhibition diameter.

From the results obtained in both antagonism tests, the FM4.C1.2 strain stands out above the others, since in both tests the strain inhibited the growth of the three pathogens, in addition, the inhibition values of thus CFS strain against two pathogens are among those with the highest antagonistic activity with inhibition diameters of 22.50 mm for *S. aureus* and 25.50 mm for *Salmonella* sp. These results may be due to the production of different organic acids (lactic, acetic, benzoic, fatty acids); which can impede cellular activity, interfere with the conservation of the membrane potential, decrease the cellular pH and thus prevent a variety of essential metabolic functions for the pathogen (Mani-Lopez et al., 2012; Omemu and Faniran, 2011). It can also be attributed to CO_2, acetaldehyde, diacetyl, hydrogen peroxide, and low molecular weight molecules such as methylhydiatonin, mevalanolactone, reuterin, and bacteriocins (De Paula et al., 2014; Moračanin et al., 2012).

Table 4. Diameter (mm) of the inhibition zones generated by the lactic acid bacteria supernatants studied against pathogenic microorganisms.

LAB strain	*Staphylococcus aureus*	*Salmonella* sp.	*Listeria monocytogenes*
CM3.C2	20.00±7.07 ab	10.00±7.07 ab	9.75±7.42 ab
FM4.C1.2	22.50±3.54 a	25.50±6.36 a	9.00±1.41 ab
CM1.C1	25.00±0.00 a	-	8.00±0.00 ab
DCM2.C3	12.50±3.54 b	-	10.00±0.00 ab
DCM3.C1.2	12.50±3.54 b	8.50±7.78 ab	-
FM1.C3	18.50±4.95 ab	-	12.50±3.54 ab
QM1.C5	-	25.00±4.24 a	14.00±2.83 ab
QM1.C5.1	-	11.00±1.41 ab	15.00±1.41 a
CM1.C3	-	-	7.25±3.89 b
CM4.C2	-	20.00±0.00 ab	-
DCM1.C1	-	26.50±4.53 a	-
DCM3.C1.1	-	2.50±0.71 b	-
DCM3.C3	22.50±3.54 a	-	-
FM1.C6.1	-	-	12.00±2.83 ab
FM2.C2	-	25.00±0.85 a	-
FM3.C1	-	-	13.00±2.83 ab
FM4.C1.1	-	-	11.00±1.41 ab
QM1.C3	12.50±3.54 b	-	-
QM2.C6	-	15.00±1.41 ab	-
QM4.C1	-	19.00±1.41 ab	-
QM4.C3	-	26.00±7.07 a	-

–: No inhibition was observed. [a-b] Equal letters per column denote significant equality (Fischer's least significant difference, $\alpha = 0.05$)

A phenomenon observed in this study is the fact that one CFS inhibited the three pathogens while the strain from which the CFS originated in direct confrontation inhibited two pathogens. This may be due to the fact that the production of active substances could vary depending on the growth conditions (agar for the strain and broth to obtain the CFS), such as oxygen concentration, pH, temperature, water activity and the nutrients; and, in this way, modify the population density, affecting the expression of genes (*quorum sensing*) that encode the production of fatty acids and bacteriocins (Beristain-Bauza et al., 2016; Sip et al., 2012).

4. Conclusion

From cheese samples it was possible to isolate lactic acid bacteria (LAB) with antagonistic capacity against pathogenic bacteria. The cell-free supernatants of some LAB were equally efficient in controlling the growth of pathogens. The strains encoded as FM4.C1.2 and CM3.C2 are potential pathogen controllers since they inhibited the three evaluated pathogens. It is convenient to evaluate the effect of the strains or their supernatants in situ (in cheeses) to validate the antagonistic effect.

Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses

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