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Food-associated lactic acid bacteria with antimicrobial potential from traditional Mexican foods

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ABSTRACT. This work was conducted to identify indigenous LAB capable of antimicrobial activity, present in traditional Mexican-foods with potential as natural preservatives. A total of 27 artisan unlabeled Mexican products were evaluated, from which 94 LAB strains were isolated, and only 25 strains showed antimicrobial activity against at least one pathogen indicator microorganism. Most of the inhibitory activity showed by the isolated LAB strains was attributed to pH reduction by organic acids. *Lactobacillus* and *Lactococcus* strains were good acid producers, depending on the substrate, and may enhance the safety of food products. Cell free cultures of *Leuconostoc mesenteroides* CH210, and PT8 (from chorizo and pulque, respectively) reduced the number of viable cells of enteropathogenic *E. coli* in broth system. *Lb. plantarum* CC10 (from "madre" of vinegar) showed significant inhibitory effect against *S. aureus* 8943. *E. faecium* QPI1 (from panela cheese) produced a bacteriocin with wide anti-*L. monocytogenes* activity. Selected LAB from traditional Mexican foods showed good potential as bio-preservatives.

Key words: Lactic acid bacteria, Mexican traditional foods, bacteriocins, *Listeria monocytogenes.*

RESUMEN. En este trabajo se identificaron bacterias ácido lácticas (BAL), presentes en alimentos tradicionales mexicanos, con potencial uso como agentes antimicrobianos naturales. Se evaluaron un total de 27 alimentos artesanales mexicanos sin marca, aislándose 94 cepas BAL, 25 de las cuales mostraron actividad antimicrobiana contra al menos un microorganismo patógeno indicador. La actividad inhibitoria mostrada por las cepas BAL aisladas, se atribuyó principalmente a la reducción del pH por la producción de ácidos orgánicos. *Lactobacillus* y *Lactococcus* fueron buenos productores de ácido, dependiendo del sustrato y podrían contribuir a la inocuidad de los alimentos. Los cultivos libres de células de *Leuconostoc mesenteroides* CH210 y PT8 (aisladas de chorizo y pulque respectivamente) lograron reducir el número de células viables de *E. coli* enteropatogénica (EPEC) en sistema en caldo. *Lb. plantarum* CC10 (aislada de madre del vinagre) inhibió significativamente a *S. aureus* 8943. *E. faecium* QPI1 (aislado de queso panela) produjo una bacteriocina con amplia actividad contra *L. monocytogenes.* Algunas BAL aisladas de alimentos tradicionales mexicanos, poseen potencial como bioconservadores.

Palabras clave: Bacterias ácido lácticas, alimentos tradicionales Mexicanos, bacteriocinas, *Listeria monocytogenes.*

INTRODUCTION

Mexican artisan foods and beverages are prepared using traditional methods, which harbor indigenous microorganisms surviving in a highly competitive microbial environment, and it is likely to find strains of lactic acid bacteria (LAB) with antimicrobial properties. The indigenous LAB present in these products contributes to their preservation due to the production of organic acids, carbon dioxide, ethanol, diacetyl, hydrogen peroxide, and bacteriocins.[20]

Examples of these traditional products include pulque, which is an alcoholic, non distilled fermented beverage, produced from the sugary sap known as aguamiel, which is extracted from different species of maguey (*Agave atrovirens, A. mapisaga, and A. salmiana*).[15] Tepache is usually produced by fermentation of pineapple mixed with water, piloncillo (a kind of brown sugar), and spices like cinnamon, green bell pepper, etc., using wood barrels.[19] The shelf life of these beverages is limited to some days. The artisan production of vinegar involves fermentation of a sugary solution by "madre", a floating yellowish mucilaginous substance, probably containing a microbial consortium including yeasts, lactic acid bacteria and *Acetobacter* spp. leading mainly to acetic acid production.

Mexican-style cheeses such as panela and ranchero, are white curd-like products having high moisture content.[35] At artisan level these cheeses are usually made from raw milk, without a starter culture, show a shelf life between one and two weeks under refrigeration,[35] and are usually consumed without any further heat processing. Mexican chorizo is a ground pork sausage mixed with garlic, chili pepper and spices.

The limited shelf life of these Mexican products makes commercialization difficult, but an appropriate strategy of food preservation could improve it. The usefulness of LAB and natural antimicrobial compounds as part of a preservation technology implies strains selection and a detailed

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study of their technological properties and limitations.[12] The aim of this study was to identify indigenous LAB capable of antimicrobial activity, present in traditional Mexican-foods as valuable technological resources to be exploited to improve microbial food safety and quality.

MATERIALS AND METHODS

Indicator strains and growth conditions

S*taphylococcus* (*S.*) *aureus* 8943, 8855, *Salmonella* 02, *Enterobacter* spp. 9476, non-01 *Vibrio* (*V.*) *cholerae* 5921, and *V. parahaemolyticus* 10691, were donated by the Queretaro's Public Health Laboratory, Mexico. *Bacillus* (*B.*) *cereus* ATCC 11778, enteropathogenic *Escherichia coli* (EPEC), *Lactococcus* (*Lc.*) *lactis* NCDO 496, *Leuconostoc* (*Ln.*) *mesenteroides* NCDO 523, *Lactobacillus* (*Lb.*) *plantarum* 5T, *Pediococcus* (*P.*) *acidilactici* ATCC 8092, and *Listeria* (*L.*) *monocytogenes* G7, and G19, were provided by the Dept. of Food Research and Postgraduate Studies, UAQ, México. *L. monocytogenes* Scott A, 19112, 7644, LCDC, 15313, SLCC 5764, 1370, and 10403S were provided by the Food Microbiology Laboratory, University of Illinois at Urbana Champaign (UIUC), USA. All LAB were grown in De Man Rogosa Sharpe (MRS; Oxoid, Basingstoke, England) medium at 30º C. Pathogenic bacteria different from *Listeria* spp. were grown in Brain Heart Infusion (BHI; Bioxon, Cuautitlán, Mex.), while *L. monocytogenes* strains were grown in tryptic soy broth (TSB; Bioxon), all at 37º C.

Isolation and screening of LAB strains with inhibitory activity

Home-made, traditional unlabeled products both from vegetable and animal origin were selected from public markets of villages within the states of Querétaro and Hidalgo. Appropriate dilutions of the food samples were plated in MRS/pimaricin (10 µg/ml) agar plates, and incubated anaerobically for 48 h, at 30º C. Colonies were picked and transferred onto MRS plates to verify purity. Gram-positive, catalase negative, non motile cells were presumptively identified as LAB. The LAB were checked for inhibitory activity by spot on the lawn test.[22] Two µl of an overnight MRS broth culture of each strain were spot-inoculated onto the surface of Trypticase soy agar (TSA, Bioxon), plus 0.5% of yeast extract (YE, Bioxon). Incubation was conducted at 30º C for 18 h in anaerobic conditions. After colonies growth, 9 ml of soft agar (0.8%) containing 10^5-10^6 CFU/ml of indicator strain were overlaid and incubated overnight. Clear zones around the colonies of LAB isolates were considered positive reaction. Isolates not showing clear zones were discarded.

Identification of LAB

The isolated LAB strains showing antimicrobial activity were identified by Gram stain, catalase production, 6.5% NaCl tolerance, gas production from glucose, growth at 10º and 45º C,[32] and by testing carbohydrate fermentation using the API 50CHL system (BioMerieux, Marcy I'Etoile, France). The QPI1 strain genotypic identification based on 16S rRNA gene sequence amplification was carried out using the primer pair 1RL-2RR.[27] Sequence alignment was conducted using the BLAST software from the Gen Bank.

Inhibitory activity by well diffusion assay

Strains showing antagonistic effect by spot on the lawn (agar plated in overlay assays) were further tested by well diffusion assay,[18] using *S. aureus* 8943, *L. monocytogenes* Scott A and non-01 *V. cholerae* 5921 as indicator microorganisms. The cell-free cultures (CFC) of LAB were produced in MRS broth by incubating for 18 h at 30° C, followed by centrifugation and filter sterilization. Two types of CFC were evaluated, with and without pH adjustment to pH = 6.5.

Acidifying activity

The acidifying activity of LAB isolates was evaluated by pH measurement. Overnight LAB cultures in MRS broth were inoculated in ultra heat-treated semi-skim milk (UHT-SM; pH 6.5), MRS broth (pH 6.2), and Trypticase soy broth (TSB; Bioxon; pH 7.1), using an inoculum of 10 ml/l.[10] All cultures were incubated at 30°C, and the pH was measured after 18 h, except for UHT-SM culture where pH was also measured after 6 h. A control of MRS and UHT-SM without inoculum was carried out at the same incubation conditions.

Inhibitory activity in broth system

The CFC of selected LAB was tested against 10^6 CFU/ml of EPEC or *S. aureus* 8943 by incubating for 5 h, at 37º C. Two controls were carried out, a growth control in TSB without CFC, and a lactic-acetic control (80 mM lactic plus 15 mM acetic acids, in 0.85% NaCl, pH 4.4). The total acids in the CFC were determined by titration with standard sodium hydroxide, and expressed as lactic acid equivalent (LAE).

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Partial characterization of bacteriocin from QPI1 strain

The sensitivity of the QPI1 CFC (400 AU/ml) to proteases was determined by incubation with each one of the following: proteinase K (EC 3.4.21.64; Sigma), trypsin (EC 3.4.21.4; Sigma), pepsin (EC 3.4.23.1; Sigma) and α-chymotrypsin (EC 3.4.21.1; Sigma), to a final concentration of 1 mg/ml.

The antimicrobial activity of semipurified fractions was conducted by the well diffusion assay using *L. monocytogenes* 7644 (10^5 UFC/ml) as indicator strain. Serial twofold dilutions of the fractions were added to the wells and the plates were incubated overnight at 37° C. The antimicrobial titer was defined as the reciprocal of the highest dilution exhibiting complete inhibition of the indicator lawn, and was expressed as arbitrary units (AU) per milliliter.

E. faecium QPI1 CFC was precipitated with 80% saturation ammonium sulfate, dialyzed, freeze dried, and labeled as FDC. Sixteen hundred arbitrary units (AU/ml) of FDC were tested in broth system against 10^5 CFU/ml of *L. monocytogenes* Scott A by incubating for 0, 1, 3, 5, and 7 h at 37º C, pH 5 and 6. The anti-listerial spectrum of freeze dried CFC was conducted by well diffusion assay using the ten *L. monocytogenes* strains mentioned in the indicator strains section.

Solid phase extraction with Sep Pak cartridges C18 (Waters, MA, USA) was used to purifiy the bacteriocin from FDC. Two active fractions were obtained, one eluting with 40% (I40), and the other with 80% (I80) isopropanol. Fraction I40 was subjected to cation exchange chromatography using a 5 mL HiTrap SP HP column (Pharmacia, Uppsala, Sweden), attached to a FPLC equipment (Pharmacia). The column was equilibrated with 100 mM sodium acetate buffer pH 4.2, at a flow rate of 2 ml/min. Elution was accomplished by a linear gradient of 50 mM sodium phosphate buffer pH 6.4, containing 1 M NaCl, for 40 min, and 3 mL fractions were collected. The semipurified fractions were analyzed by Tricine-SDS-PAGE, using 16.5% acrylamide,[31] and the peptide bands were identified by staining with Coomassie Blue dye.

RESULTS

Isolation and screening for inhibitory activity

A total of 27 artisan Mexican foods were evaluated, 17 of which were of animal origin and included panela and ranchero cheeses, as well as chorizo. The remaining 10 foods were of plant origin: pulque, tepache, and "madre" for vinegar production. From these 27 foods, 94 LAB strains were isolated, but only 25 strains showed antimicrobial activity against at least one pathogen indicator, as tested by the spot on the lawn method (Table 1). Five of these isolates were identified as *Lc. lactis* subsp. *lactis*, fourteen as *Lb. plantarum,* one as *Lb. delbruekii,* two as *Lb. paracasei* subsp. *paracasei,* two as *Ln. mesenteroides* and one as *E. faecium.*

The genotypic identification from the 1570 bp complete 16S rDNA gene of strain QPI1 permitted identity confirmation with a 99% homology as *E. faecium*, when compared to a strain with accession No. AY172570. Twenty one out of twenty five LAB strains were able to inhibit *L. monocytogenes*, regardless the origin of the fermented food. Mainly animal origin LAB (four out of six strains) inhibited *S. aureus* strains. Ten out of thirteen LAB that inhibited *Vibrio* spp. were *Lactobacillus* strains (Table 1).

The CFC from *E. faecium* QPI1, isolated from panela cheese, was the only strain that kept inhibitory activity in both tested methods: spot on the lawn, and well diffusion with pH adjustment (Fig. 1).

The freeze dried CFC from *E. faecium* QPI1 showed inhibition of *L. monocytogenes* 7644 using 25 AU/ml, *L. monocytogenes* 1370 and 10403S using 100 AU/ml, *L. monocytogenes* 19112, LCDC, 15313, SLCC 5764, and G7 using 200 AU/ml, *L. monocytogenes* G19 using 400 AU/ml and *L. monocytogenes* Scott A using 800 AU/ml; exhibiting its wide potential as bio-preservative.

Acidifying capacity

The acidifying capacity of the four LAB genera isolated in this work, growing in three different culture media are shown in Figure 2. *Lactobacillus* strains grown in MRS showed a pH reduction of 2.3 ± 0.16 units significantly higher ($p < 0.05$) than LAB from other genera tested, to a mean final pH of 3.9 ± 0.2. Same pattern was observed when grown in TSB, with a significant pH reduction ($p < 0.05$) of 1.8 ± 0.07 units to a pH = 5.3 ± 0.07. However, when UHT-SM was used as fermentation medium, *Lactococcus* strains showed a pH reduction of 2.0 ± 0.6 with final pH of 4.7 ± 0.6, significantly different from *Lactobacillus* strains which only reduced 0.65 ± 0.55 pH units to a final pH of 5.9 ± 0.6 ($p < 0.05$).

Inhibitory activity in broth system

The acidifying capacity showed by the LAB isolates suggested their potential for pathogen growth inhibition. Four strains showing the largest inhibition zone and wide spectra as tested by the spot on the lawn (Table 1), were chosen for evaluation in broth system: *E. faecium* QPI1 (0.7% LAE), *Lb. plantarum* CC10 (1.7% LAE), *Ln. me-*

Table 1. Inhibitory spectra of LAB isolated from artisan Mexican foods by spot on the lawn test.

	Isolates	*Indicator strains*														
		S. aureus 8943	*S. aureus* 8855	*L.*[a] 7644	*L.*[a] Scott A	*L.*[a] *G19*	*B.*[b] 11778	*Lc.* 496	*Ln.* 523	*Lb.* 5T	*P.* 8092	EPEC	*Salmonella* 02	*Enterobacter* 9476	*V.*[c] 5921	*V.*[d] 10691
Animal origin foods																
E. faecium	QPI1	++	++	+++	+++	+++	-	-	-	-	++	-	-	-	-	-
Lc. Lactis	QPII4	-	-	++	++	++	-	-	-	-	++	-	-	-	-	-
	QPIII6	-	-	++	++	++	-	-	-	-	++	-	-	-	-	-
	QMIV3	-	-	++	++	++	-	-	-	-	++	-	-	-	-	-
	QPI3	-	-	++	++	+	-	-	-	-	+	-	-	-	-	-
	QO14	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+
Lb. plantarum	QMIV1	-	-	++	++	++	-	-	-	-	-	-	-	-	-	-
	QMIV6	-	-	++	++	++	++	-	-	-	-	-	-	-	-	-
	QMIV7	-	-	++	++	++	-	-	-	-	-	-	-	-	-	-
	CS1	-	-	++	++	++	-	-	-	-	+	-	-	-	-	-
	CHO1	+	+	-	-	-	-	-	-	-	+	-	-	-	++	++
	CHO10	-	-	++	+++	++	-	-	-	-	+	-	-	-	+	+
Lb. delbruekii	CHI4	-	-	-	++	++	-	-	-	-	+	-	-	-	-	-
Ln. mesenteroides	CH210	++	+++	++	++	++	-	-	++	-	-	-	-	+	++	++
Plant origin foods																
Lb. paracasei	VN4	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-
	VN7	-	-	-	-	-	-	-	-	-	++	-	-	-	++	++
Lb. plantarum	CC9	-	-	++	++	+	++	-	-	-	-	-	-	-	-	++
	CC10	-	-	++	++	++	++	-	++	-	-	-	-	-	++	++
	TPI13	-	-	++	++	++	-	-	-	-	++	-	-	-	++	++
	TII18	-	-	++	-	++	-	-	-	-	++	-	-	-	++	-
	TII19	-	-	++	-	++	-	-	-	-	-	-	-	-	++	++
	PII2	-	-	++	++	++	-	-	-	-	++	-	-	-	-	-
	CTU3	-	-	++	++	-	++	-	-	-	++	-	-	-	-	-
	CTU4	-	+	++	++	-	++	-	-	-	-	-	-	-	++	++
Ln. mesenteroides	PT8	+	++	+	+++	+	-	-	-	-	-	-	-	-	++	++

(-) no inhibition, (+) 0.5-2.0 mm diameter of inhibition, (++) 2.1-8.0 mm diameter of inhibition, (+++) > 8.0 mm diameter of inhibition

[a] *L.* = *Listeria monocytogenes*

[b] *B.* = *Bacillus cereus*

[c] *V.*= non-01 *V. cholerae*

[d] *V.* = *V. parahaemolyticus*

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senteroides CH210 (1.0% LAE), and *Ln. mesenteroides* PT8 (1.2% LAE). Table 2 shows the decrease of viable cells of *S. aureus* 8943 or EPEC when CFC of these four strains are exposed to 10^6 CFU/ml.

The CFC from *Lb. plantarum* CC10 killed nearly all cells of both *S. aureus* and EPEC, showing that the antimicrobial effect was associated to acid production (Table 2), which resulted in a low pH (3.7) of the CFC. At this pH about 20% of lactic acid and 90% of acetic acid remained undissociated (form responsible for most antimicrobial effect), which did not occur for the other three CFC (final pH = 4.2-4.7).

A high viable cells reduction of EPEC in broth system (more than 5 log cycles), was achieved by the CFC of *Ln. mesenteroides* PT8 (final pH 4.4). Since *S. aureus* 8943 population was reduced to a lower extent (2.85 log cycles) by this CFC, the antimicrobial effect might depend not only on the acid production, but also on the microorganism sensitivity.

The inhibitory activity of the CFC from *E. faecium* QPI1 against *S. aureus* 8943 was similar to that showed by the acid control, despite its low % LAE, suggesting the effect of its bacteriocin.

Partial characterization of bacteriocin from QPI1 strain

Proteinase K treatment did not show any effect on the antimicrobial activity of QPI1 extracts, while 50% activity

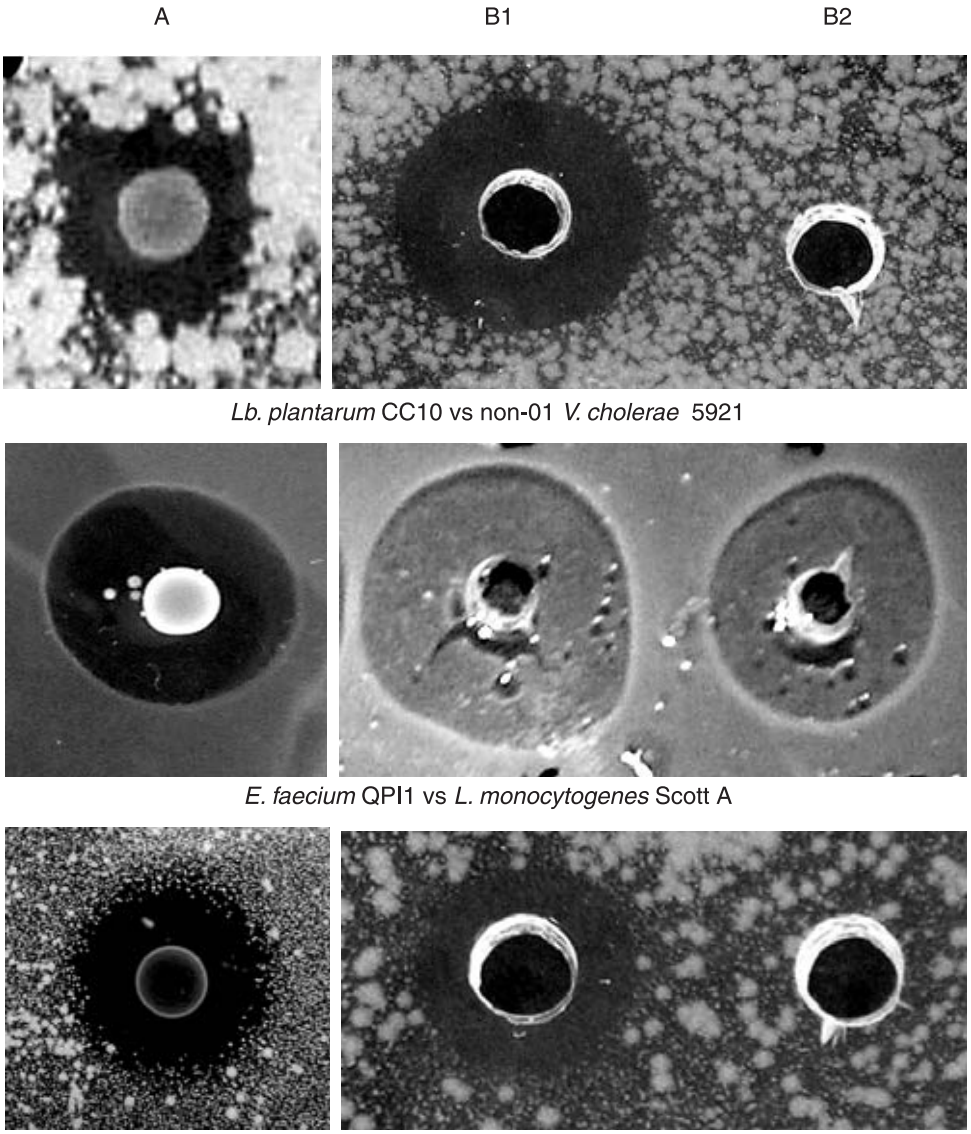

Figure 1. *A: Spot on the lawn test. B: Well diffusion assay (WDA) vs cell free extract of the tested LAB strains. B1, WDA without pH adjustment. B2, WDA with pH adjusted to 6.5.*

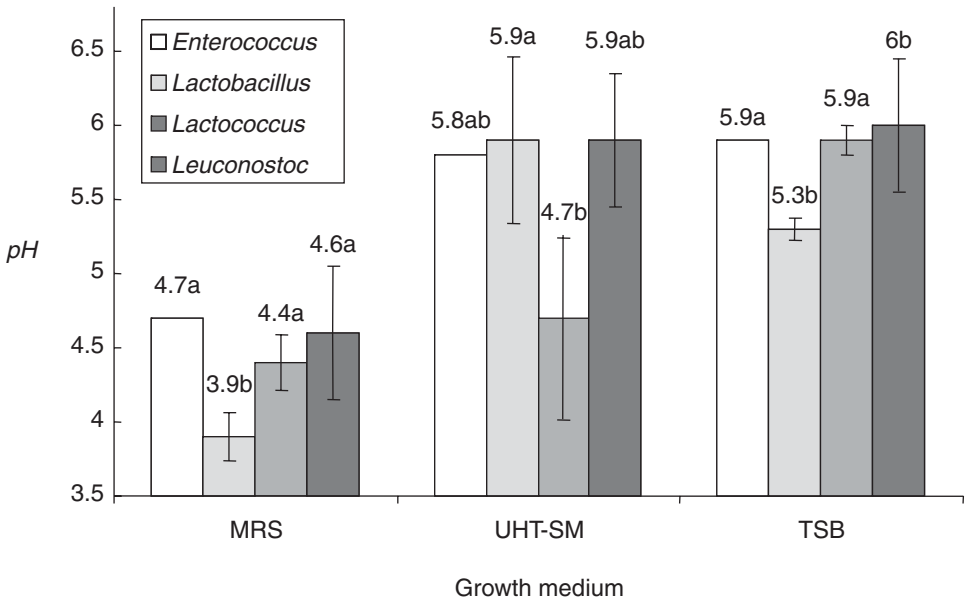

Figure 2. *Mean pH value reached after growth of the corresponding LAB strain (classified by genus) in MRS (DeMan Rogosa Sharpe Broth), UHT Semi-Skim Milk, and Trypticase Soy Broth (TSB) media. Mean values were compared using the Tukey-Kramer test, and those not connected by same letter are significantly different ($p < 0.05$).*

reduction resulted after treatment with both pepsin and trypsin. Antimicrobial activity was completely lost upon treatment with α-chymotrypsin, confirming the protein nature of the antimicrobial compound in the CFC. Additionally, heat exposure at 80° and 100° C for 20 min produced no effect on the inhibitory activity.

Inhibitory activity of FDC from *E. faecium* QPI1 against *L. monocytogenes* Scott A was observed at two pH values, in broth system (Fig. 3). The four log reduction of initial viable cells number at pH 5 was higher than that at pH 6 (2.4 log reduction).

Cation exchange chromatography of the I40 fraction showed an active peak (FP-I40) which eluted at 30% NaCl (results not shown). Fractions I80, and FP-I40 showed little antimicrobial activity when tested separately. However, a mixture of both semi-purified fractions gave full antimicrobial activity, indicating a synergistic effect. Fraction I80 showed a purification fold of 3.43 (5924 AU/mg protein), while purification fold of fraction FP-I40 was 5.67 (9780 AU/mg protein). Broad single bands were obtained after electrophoresis of each fraction (Fig. 4), showing a molecular weight between 2 and 4 kDa, suggesting good purity but lack of homogeneity, and further purification experiments are required.

DISCUSSION

About 26.6% of the isolated LAB strains were capable of inhibitory activity, but only one strain (1.0%) showed bacteriocin production capacity, in agreement with similar reports.[1,26,33] Most inhibitory activity was attributed to organic acids produced by the LAB isolates, since a pH adjustment of their CFC resulted in loss of inhibitory activity.

Table 2. Effect of cell free culture (CFC) produced by LAB on the cell viability of *S. aureus* 8943 and enteropathogenic *E. coli* (EPEC). 10^6 CFU/mL were incubated for 5 h at 37° C with the corresponding CFC or control.

Treatment	Log CFU reduction[a] *S. aureus* 8943	EPEC
Growth control (TSB)	-1.7 ± 0.14	-2.55 ± 0.12
Lactic-Acetic Control[b]	4.25 ± 0.49	4.30 ± 0.14
Lb. plantarum CC10 CFC	6.20 ± 0.14	6.63 ± 0.08
Ln. mesenteroides PT8 CFC	2.85 ± 0.35	5.88 ± 0.34
Ln. mesenteroides CH210 CFC	1.95 ± 0.07	1.45 ± 0.21
E. faecium QPI1 CFC	3.90 ± 0.14	1.41 ± 0.01

[a] $\log_{10}$ CFU at time = 0 minus $\log_{10}$ CFU at time 5 h.
[b] Mixture of 80 mM lactic acid, plus 15 mM acetic acid, adjusted to pH 4.4
Data represent the means of three determinations ± standard deviation.

The spot test inhibitory spectra of the LAB isolates was characterized by inhibition of Gram positive pathogenic bacteria, such as *S. aureus* and *L. monocytogenes,* and the Gram negative non-01 *V. cholerae* and *V. parahaemolyticus.* These are pathogens of human health significance, which have been implicated in outbreaks from Mexican foods.[14]

The culture medium used to grow the tested LAB showed clear effect on their acidifying activity. Lactobacilli strains showed the highest pH reduction (2.3 ± 0.16

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units) in MRS (2% glucose), while only 0.65 ± 0.55 pH units reduction when growing in UHT-SM (4.8% lactose), despite its lactose fermentation capacity (result not shown). A similar pH reduction was achieved by Lactococci in both MRS (1.8 ± 0.2) and UHT-SM (1.8 ± 0.7), showing its versatility in organic acids production. The pH reduction resulting from the acidifying activity was attributed to the amount and type of organic acids produced, which varies according to the carbohydrate source.[8] Thus, specific LAB strains must be carefully selected for every food system to promote *in situ* acid production, as part of a biopreservation method.[21]

Ninety percent of LAB isolates from plant origin foods were Lactobacilli genus, which have been associated with Mexican fermented products of plant origin.[15,16] *Lb. plantarum* CC10 showed high viability reduction against *S. aureus* and EPEC in broth system, and showed antimicrobial activity against *L. monocytogenes* and non-01 *V. cholerae* in spot test. This suggested its importance as part of combined methods for food preservation,[21] in fermented vegetable products like tepache and pulque.

Lactobacillus paracasei VN4 and VN7 isolated from 'madre', showed capacity to ferment the nondigestible polysaccharide inulin, this quality has been associated to probiotic bacteria.[25]

Lactococci isolates showed significantly higher acidifying activity in UHT-SM than that showed by lactobacilli strains, in agreement with previous reports.[6,24] *Lc. lactis* subsp. *lactis* QPII4, QPIII6 and QO14 were fast acid producers, since the pH in UHT-SM was reduced to below 5.3 after 6 h of incubation (results not shown). This property is a required characteristic for starter cultures, and could play an important role in cheese manufacture.[6,10] *Lc. lactis* subsp. *lactis* is considered the most important *Lactococcus* species in cheese fermentation, and has been frequently isolated from raw milk cheeses.[7,23] The above mentioned lactococci strains may be added as part of the hurdle technology system for the preservation of Mexican-style cheeses.

Two *Ln. mesenteroides* PT8 (from pulque) and CH210 (from chorizo) showed inhibitory properties in spot agar and in broth system. The activity was lost in antagonism experiments with pH adjustment, showing that it was associated to organic acids and carbon dioxide. The effect of H_2O_2 was considered less significant since this metabolite is not stable in MRS broth.[29] Leuconostoc as an obligately heterofermentative bacterium, might have showed enhanced antimicrobial activity due to the production of CO_2 and ethanol, besides organic acids.[11] Leuconostoc was effective in the biopreservation of vacuum-packed meat, and cooked meat products,[5] and has also been associated to the thickening of Mexican pulque.[15] Thus, Leuconostoc isolates could be used to improve the safety of pulque and meat products like chorizo.

E. faecium QPI1 showed poor acid production in every growth medium tested in agreement with previous studies.[3,6] However, significant inhibitory activity was observed in spot test, well diffusion, and broth systems,

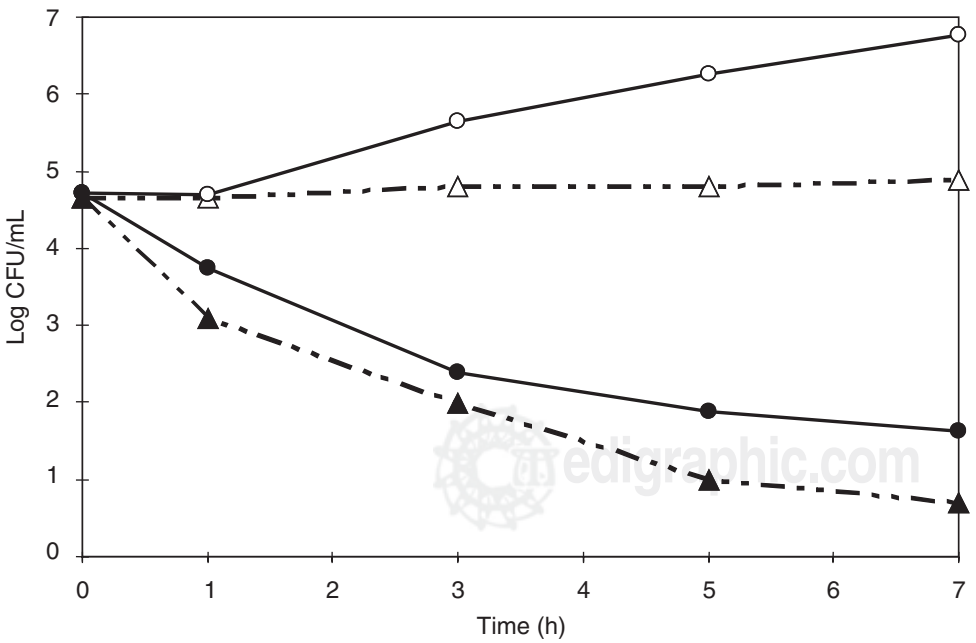

Figure 3. *Effect of E. faecium QPI1 freeze dried culture (FDC-QPII) on cell viability of* Listeria monocytogenes *Scott A. Exponential phase cells were incubated at 37°C in TSB broth at pH 6 (○, ●), and pH 5 (▲, △); without addition of FDC-QPI1 (○, △), and 1,6000 AU/mL of FDC-QPI1 added (●, ▲).*

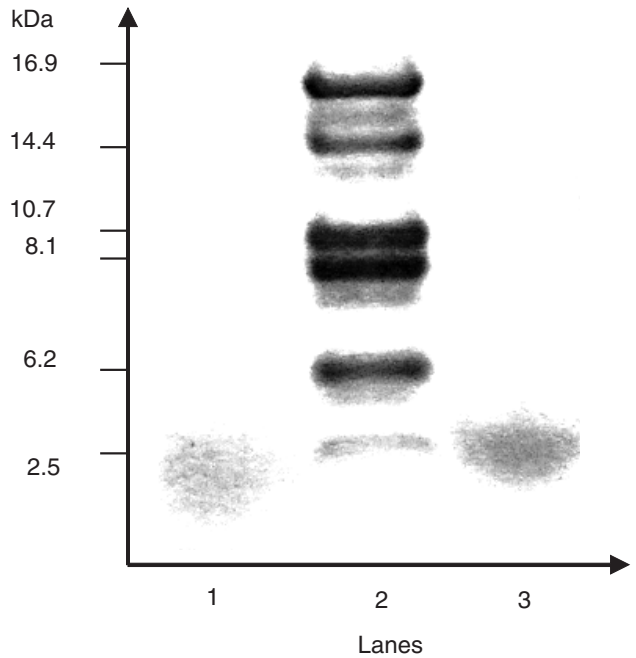

Figure 4. *Tricine-SDS-PAGE of partially purified enterocins from E. faecium UQI1. Lanes: 1, enterocin I80; 2, molecular weight markers (Amersham); 3, enterocin FP-I40.*

mainly against *S. aureus,* and *L. monocytogenes*. Bacteriocin production by this strain was confirmed because of its proteinaceous nature, heat resistance, and wide anti-listerial activity. Full antimicrobial activity was observed when the semipurified peptides contained in fractions I80 and FP-I40 were combined. Up to date only a two-peptide bacteriocin (enterocin L50A and L50B) from *Enterococcus faecium* has been reported.[9] More studies are needed to identify the two partially purified peptides reported in this work.

The bacteriocin produced by *E. faecium* QPI1 was capable to inhibit *L. monocytogenes* strains from human, animal, and food origin, including strains G7 and G19 isolated from Mexican avocado,[4] confirming its usefulness against Mexican strains.

Enterococci have been frequently found in traditional cheeses produced from raw milk.[7,23,34] They are known to play an important role in artisan cheese production, and the technological and probiotic benefits of enterococci are widely recognized.[3,30] Control of *L. monocytogenes* by bacteriocin-producing enterococci strains has shown successful results in milk and meat products.[2,17,28] This property makes the enterocins a promising means of food preservation, allowing a selective inhibition of *L. monocytogenes,* while maintaining little or no inhibitory effect on most starter and non-starter LAB used to preserve and develop flavor in foods. *E. faecium* QPI1 was not hemolytic in human or sheep blood agar, and was sensitive to vancomycin (results not shown), suggesting its safe use in food manufacture.[13] Utilization of *E. faecium* QPI1 could be of great interest in fresh cheese manufacture, because these foods are consumed without previous thermal processing, and have been implicated in food outbreaks.[14] A LAB co-culture may be used to produce bacteriocin *in situ*, or the CFC may be used as food additive.[20,34] Some selected LAB strains, such as *Lc. lactis* QPII4, QPIII6, QO14, and *E. faecium* QPI1, may be used as starters in large-scale industrial processes involving pasteurized milk, to improve their quality and safety.

LAB originally isolated from traditional foods are probably the best candidates for improving the microbiological safety of these foods, because they are well adapted to those environments and should therefore, be more competitive than starters or LAB from other sources.

Our results indicate that some selected food-associated LAB show antimicrobial potential which can be useful in food preservation technology. More studies are necessary to establish appropriate conditions such as pH, temperature, target microorganisms, and substrate, to achieve full antimicrobial activity.

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