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Selection of EPS-producing *Lactobacillus* strains isolated from kefir grains and rheological characterization of the fermented milks

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ABSTRACT

In this study the ability to produce exopolysaccharides during growth in milk of 28 *Lactobacillus*, previously isolated from kefir grain, was investigated and the rheological properties of the obtained fermented milks were also studied. During fermentation all microorganisms were able to produce polysaccharide with final concentration ranging from 20 mg L^{-1} to 370 mg L^{-1}. *Lactobacillus kefiranofaciens* and *Lactobacillus plantarum* strains produced oligosaccharide with low degree of polymerization. The exopolysaccharides produced by the five *Lactobacillus paracasei* strains presented also a high molecular weight fraction. In consequence the acid milk gels obtained by fermentation with all the *L. paracasei* strains were the ones that presented the highest viscosities. Nevertheless the viscolestic characteristics of the resulting acid gels were different. *L. paracasei* CIDCA 83123 produces a milk having a viscous behavior whereas fermented milks with *L. paracasei* CIDCA 8339, CIDCA 83120, CIDCA 83121 and CIDCA 83124 has gel structure.

The five *L. paracasei* strains isolated from kefir studied in the present work were the first described that produce high molecular weight exopolysaccharides. Since production of high molecular weight exopolysaccharides affects the viscosity of fermented milk these strains could have a successful application improving texture of fermented dairy product.

1. Introduction

Exopolysaccharides (EPSs) produced by lactic acid bacteria (LAB) are extensively studied biomolecules of great interest for food, medical and pharmaceutical industry. They exhibit heterogenous composition and structure and in consequence they have broad range of physicochemical properties (Piermaria, de la Canal, & Abraham, 2008; Ruas-Madiedo, Abraham, Mozzi, & de los Reyes-Gavilán, 2008). In food industry they contribute to the rheology, mouthfeel and texture of fermented milks, cheese or baked products (Galle et al., 2012). Additionally, several exopolysaccharides are reported to function as health promoters. Among the beneficial effects attributed to these biopolymers it can be mentioned cholesterol lowering capability, immunomodulating ability, intestinal epithelium protection of the action of pathogen microorganisms or faecal microbiota modulation capability (Nicolic et al., 2012; Ruas-Madiedo et al., 2008; Salazar, Ruas-Madiedo, Prieto, Calle, & de los Reyes-Gavilán, 2012).

Kefir is fermented milk produced with an original native starter, the kefir grains. The grains are macroscopic structures composed of protein and polysaccharide named kefiran that encompass a complex microbiota represented by LAB (primarily *lactobacilli* and *lactococci*), yeasts and acetic acid bacteria that coexist in symbiotic association (Garrote, Abraham, & De Antoni, 2010). Kefiran is a branched hydrosoluble glucogalactan composed by equal amounts of glucose and galactose whose production could be attributed to *Lactobacillus kefiranofaciens* (Ahmed, Wang, Anjum, Ahmad, & Khan, 2013; Vancanneyt et al., 2004). This EPS has the ability to improve the texture of fermented products (Rimada & Abraham, 2006; Yovanoudi, Dimitreli, Raphaelides, & Antoniou, 2013) and exhibits advantageous biological properties, such as inmunostimulation, anti-tumor and anti-ulcer activities and epithelium protection (Medrano, Racedo, Rolny, Abraham, & Pérez, 2011;

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Murofushi, Mizuguchi, Aibara, & Matuhasi, 1986; Vinderola, Perdigon, Duarte, Farnworth, & Matar, 2006).

Besides to *L. kefiranofaciens* some strains of lactic acid bacteria and yeast isolated from kefir are capable to produce exopolysaccharides different to kefiran in pure culture (Frengova, Simova, Beshkova, & Simov, 2002; Jiang, Qian, Ren, & Mu, 2013; Liu, Xie, Han, & Zhang, 2013; Wang et al., 2010; Zhou et al., 2014). Kefir grains, therefore, are an important source of EPS producer microorganisms capable to improve the textural properties of fermented products.

It was demonstrated that fermented milks prepared with EPS-producing cultures showed increased ropiness and tended to be creamier than yoghurts without these cultures (Folkenberg, Dejmek, Skriver, Guldager, & Ipsen, 2006). Within the complex community of kefir grain several strains of *Lactobacillus plantarum*, *Lactobacillus kefir*, *Lactobacillus parakefir*, *Lactobacillus paracasei* and *L. kefiranofaciens* were isolated in our group (Garrote, Abraham, & De Antoni, 2001; Hamet et al., 2013) and only *L. kefiranofaciens*, *L. plantarum*, and *L. paracasei* strains are able to grown in milk and could be used for dairy fermentation. The aim of this work was to evaluate the ability of lactobacilli isolated from kefir grain from CIDCA collection to produce exopolysaccharides in milk, determine the molecular weight distribution of the exopolysaccharides produced for each strain and to analyze the rheological properties of the fermented milks in order to select microorganism for the application in fermented milk based foods.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Twenty eight different strains of *Lactobacillus* isolated from kefir belonging to the species *L. plantarum* (14 strains), *L. kefiranofaciens* (9 strains) and *L. paracasei* (5 strains) were used.

L. plantarum strains were grown in aerobic conditions in MRS broth (Difco Laboratories, Detroit, MI, USA) at 30 °C for 24 h. *L. kefiranofaciens* strains were cultured in MRS broth pH 5.0 (MRS acidified with HCl to reach pH 5.0) in anaerobic jars using AnaeroPack-Anaero (Mitsubishi Gas Chemical CO, Inc. Tokyo) at 30 °C for 7 days. *L. paracasei* strains were grown in MRS broth in aerobic conditions at 30 °C during 48 h.

Before each experiment, strains were subcultured in commercial ultra-high temperature (UHT) low fat milk obtained from Sancor (Santa Fe, Argentina) and incubated at the same conditions described above for each specie to obtain fresh pure cultures.

2.2. Preparation of fermented milk

Ten milliliters of fresh pure cultures in milk of each microorganism, containing 10^8 colony forming units per milliliter (*CFU* mL^{-1}), were inoculated to 1 L of commercial UHT low fat milk (Sancor, Santa Fe, Argentina) and incubated in the conditions described in Section 2.1. Chemically acidified milk was used as control of acid milk gel. It was obtained by addition of glucono-δ-lactone (ICN Biomedicals Inc., Ohio 44202, USA) to milk at a concentration of 10 g L^{-1} and incubation at 37 °C for 3 h.

2.3. Isolation and purification of polysaccharides from fermented milk

A volume of 100 mL of fermented milk was heated in a boiling water bath for 30 min with discontinuous stirring in order to dissolve the polysaccharide attached to cells and to inactivate the enzymes that could hydrolyze the polymer. Cells were removed by centrifugation at 10000 *g* for 20 min at 20 °C in a Avanti J25 centrifuge (Beckman Coulter Inc., Palo Alto, California). The polysaccharide in the supernatant was precipitated by addition of two volumes of cold ethanol and left at −20 °C overnight. Then, samples were centrifuged at 10000 *g* for 20 min at 4 °C. EPS pellets were dissolved in hot distilled water and dialyzed for 48 h at 4 °C against bi-distilled water through dialysis membranes molecular weight cut-off of 1 kDa (Spectra/Por, The Spectrum Companies, Gardena, CA, USA) according to Rimada and Abraham (2003). The samples were tested for the absence of other sugars or proteins by qualitative thin layer chromatography (TLC) and the Bradford method respectively. TLC was made on Silica gel G type 60 plates (Merck D-64271 Darmstadt, Germany) using n-propanol-acetic acid-water (volumetric proportion, 70:20:10) as the mobile phase. TLC plates were developed with p-amino benzoic acid 7 g L^{-1} and o-phosphoric acid 30 g L^{-1} in methanol. Bradford and thin layer chromatography reagents were obtained from Sigma (St. Louis MO 63178 USA).

2.4. Polysaccharides quantification and molecular mass estimation

Total sugars concentration in purified solution of polysaccharide was determined by the anthrone method, measuring absorbance at 620 nm (Southgate, 1976). Glucose (Sigma St. Louis MO 63178 USA) was used to prepare standard solutions.

The concentration of polysaccharide solution was adjusted to 0.5 g L^{-1} and filtered through 0.45 μm filters (Millipore, Sao Paulo, Brazil), previously to molecular mass determination. Molecular mass estimation was carried out by gel filtration using OH-PAK SB-805HQ gel filtration chromatography column (SHODEX, Kawasaki, Japan) in a HPLC system (Waters, Milford) based on the method described by Turquois and Gloria (2000). Samples of polysaccharide solutions (50 μL) were injected into the column and eluted at room temperature, using $NaNO_3$ 0.1 mol L^{-1}. The flow rate was kept constant at 0.95 mL min^{-1} (pressure 827.40 kPa–896.35 kPa). The eluant from the column was analyzed on-line by RI (refractive index) detection in a 410 differential refractomer (Waters, Milford). Dextrans with molecular masses of 97 kDa, 145 kDa, 326 kDa, 548.3 kDa, 848.2 kDa, 2370 kDa and 3800 kDa (Phenomenex, Torrance, CA) were used as standards.

2.5. Rheological characterization of fermented milk

Rheological characterization of fermented milks was performed in a Haake ReoStress 600 (Thermo Haake, Karlsruhe, Germany), in rotational and oscillatory modes. Rotational analysis was performed at 25 °C with a 1 mm gap plate–plate sensor system PP35. Shear stress was determined as a function of shear rate. An acceleration of 4167 s^{-2} was used to increase shear rate from 0 s^{-1} to 500 s^{-1} and the same but negative acceleration value to decrease shear rate until 0. Rheological behavior was correlated by Ostwald-de-Waele model:

$$\tau = k \cdot D^n$$

where τ is the shear stress (Pa), κ is the consistency index (Pa s^n), D is the shear rate (s^{-1}) and n is the flow index (dimensionless). Apparent viscosities (mPa s) were calculated at 300 s^{-1}.

Small deformation oscillatory measurements were carried out with a serrated plate-and-plate geometry (35 mm diameter, 1 mm gap). Samples were carefully removed from a vessel and placed onto the bottom plate of the rheometer. Excess sample was then removed and low viscosity silicone oil was applied to prevent evaporation. The temperature was maintained at 25 °C by a circulating water bath (DC50, Haake). The linear viscoelastic region was determined through stress sweep test at a fixed frequency (1 Hz). G'

(storage modulus) and G'' (loss modulus) were evaluated at a function of frequencies (0.1 Hz–10 Hz) within the linear range.

2.6. Statistical analysis

All experiments were performed at least in triplicate. Differences were statistically tested using Analysis of Variance (ANOVA) and Fisher's least significant difference (LSD) mean discrimination test, using $p \leq 0.05$ or $p \leq 0.01$ as levels of significance (SYSTAT software, version 10.0).

3. Results and discussion

3.1. Polysaccharide production and partial characterization of EPS

EPS-producing lactic acid bacteria are of interest in dairy industries due its contribution to rheological properties of products or because they can provide health promoting properties to the fermented food (Nikolic et al., 2012). Taking this into consideration, selection of EPS producing strains and the quantification of EPS in the fermented milk is required to know the potential application of new strains as starters (Enikeev, 2012). The EPS producing ability of *L. plantarum*, *L. kefiranofaciens* and *L. paracasei* isolated from kefir grains, grown in milk at 30 °C, was studied and the obtained EPS concentrations are shown in Table 1. During fermentation, all microorganisms were able to produce polysaccharide with a final concentration ranging from 20 mg L^{-1} to 370 mg L^{-1} being the final pHs of the fermented milks between 3.6 and 4.5 (data not shown). Sixteen strains yielded relatively large amounts of EPS, between 145 mg L^{-1} and 370 mg L^{-1}; eight strains produce EPS in the range between 80 mg L^{-1} and 145 mg L^{-1} and four strains produced less than 80 mg L^{-1}. Analyzing EPS production between species it can be noted that all the strains of *L. paracasei* produce large amount of EPS as well as 6 of the 11 strains of *L. kefiranofaciens*, whereas the production of EPS by *L. plantarum* was the most variable since some strains produced only 20.4 mg L^{-1} while other produce more than 350 mg L^{-1}. During fermentation of milk with kefir grains the EPS named kefiran is produced to reach a final concentration that ranged from 100 to 200 mg L^{-1}, depending on the culture condition (Enikeev, 2012; Rimada & Abraham, 2003). In consequence, the evaluated strains, in the condition of grown studied, produce a higher amount of EPS than corresponding to milk fermentation by whole kefir grain. Otherwise, several strains isolated from kefir grains were reported as capable of producing exopolysaccharide in pure culture. They were identified as *L. kefiranofaciens* (Micheli, Uccelletti, Palleschi, & Crescenzi, 1999) and *L. bulgaricus* (Frengova et al., 2002), that produce EPS with a sugars composition similar to kefiran; *L. helveticus*, *S. thermophiles* (Frengova et al., 2002; Jiang et al., 2013) and *L. paracasei* (Liu et al., 2013) that produce low molecular weight EPS. Among the publications mentioned above, only Frengova et al. (2002) reported EPS production in milk. Within strains studied by these authors, only two produced more than 300 mg L^{-1} of EPS.

The determination of exopolysaccharide concentration is not enough to understand the contribution of EPS producing strains to the rheological properties of a fermented product. Among physicochemical characteristic of the EPS, molecular weight distribution is one of the parameters that should be considered. The molecular weight distribution of EPS isolated from fermented milks was determined by gel exclusion chromatography and results are presented in Table 1. Most of the studied *L. kefiranofaciens* strains produced oligosaccharide with low degree of polymerization, whose molecular weight was lower than 10^4 Da. EPS produced by most of *L. plantarum* strains showed a molecular weight between 10^4 Da to 10^5 Da. EPS produced by the 5 strains of *L. paracasei* had, as a main component, a fraction of molecular weight between 10^4 Da to 10^5 Da, but a high molecular weight fraction was also observed. EPS produced by *L. paracasei* CIDCA 8339 and *L. paracasei*

Table 1

Concentration (C) and molecular weight size distribution (MSD) of polysaccharides produced by *Lactobacillus* strains isolated from kefir in milk and flow (n) and consistency index (κ) obtained from Ostwald de Waele fitted model corresponding to achieved fermented milks.

	Strain	C/(mg L^{-1})	MSD/Da				Ostwald de Waele parameters	
			$<10^4$	10^4 to 10^5	10^5 to 10^6	$>10^6$	n	κ/(Pa s^n)
L. plantarum	CIDCA 8312	116.2	0	100	0	0	0.12 ± 0.001	5.4 ± 0.2
	CIDCA 8313	171.8	0	100	0	0	0.12 ± 0.010	4.8 ± 0.3
	CIDCA 8316	105.2	0	100	0	0	0.15 ± 0.032	4.0 ± 0.6
	CIDCA 8318	180.7	0	99.8	0.2	0	0.13 ± 0.003	5.5 ± 1.7
	CIDCA 8323	20.4	0	100	0	0	0.11 ± 0.004	5.5 ± 0.4
	CIDCA 8324	45.3	0	98.8	1.2	0	0.13 ± 0.040	3.8 ± 0.6
	CIDCA 8327	160.4	0	100	0	0	0.15 ± 0.045	4.4 ± 1.0
	CIDCA 8333	32.6	0	100	0	0	0.15 ± 0.020	3.7 ± 0.5
	CIDCA 8336	301.2	91.5	8.6	0	0	0.14 ± 0.002	4.6 ± 0.2
	CIDCA 8337	370.6	0	100	0	0	0.14 ± 0.026	4.4 ± 0.8
	CIDCA 8342	166.1	0	99.1	0.9	0	0.15 ± 0.034	4.0 ± 1.7
	CIDCA 83112	72.2	96.8	3.3	0	0	0.15 ± 0.022	4.1 ± 0.2
	CIDCA 83114	100.3	0	100	0	0	0.14 ± 0.002	4.1 ± 0.2
	CIDCA 83210	122.5	0	100	0	0	0.15 ± 0.015	3.7 ± 0.5
L. kefiranofaciens	CIDCA 83118	202.4	100	0	0	0	0.09 ± 0.009	6.20 ± 0.03
	CIDCA 83119	301.9	78.2	21.8	0	0	0.07 ± 0.002	5.98 ± 1.31
	CIDCA 83122	198.2	78.3	21.7	0	0	0.05 ± 0.003	8.11 ± 2.15
	CIDCA 83211	112.9	77.5	22.56	0	0	0.04 ± 0.003	4.41 ± 0.77
	CIDCA 83212	125.9	97.7	2.3	0	0	0.15 ± 0.015	2.54 ± 0.07
	CIDCA 83310	178.9	100	0	0	0	0.03 ± 0.001	6.89 ± 0.83
	CIDCA 83311	183.2	100	0	0	0	0.15 ± 0.015	2.30 ± 0.07
	CIDCA 8351	111.9	100	0	0	0	0.07 ± 0.021	4.53 ± 0.37
	CIDCA 8371	85.1	79.7	20.4	0	0	0.20 ± 0.016	1.39 ± 0.12
L. paracasei	CIDCA 8339	145.2	0	81.6	18.4	0	0.06 ± 0.01	9.3 ± 3.1
	CIDCA 83120	198.9	0	90.3	0	9.7	0.19 ± 0.02	5.1 ± 0.3
	CIDCA 83121	234.9	0	76.7	0	23.3	0.23 ± 0.03	4.9 ± 0.6
	CIDCA 83123	159.4	0	62.9	5.7	31.5	0.07 ± 0.01	17.6 ± 0.9
	CIDCA 83124	209.3	0	76.3	21.9	1.8	0.14 ± 0.01	6.8 ± 1.8

CIDCA 83124 had a molecular weight fraction of 10^5 Da–10^6 Da. EPS produced by *L. paracasei* CIDCA 83120, *L. paracasei* CIDCA 83121 and *L. paracasei* CIDCA 83123 had a fraction with molecular weight higher than 10^6 Da. EPS produced by *L. paracasei* strains were reported by Dupont, Roy, and Lapointe (2000); Robijn et al. (1996); Xu, Ma, Wang, Liu, and Li (2010) but these authors did not evaluate the molecular weight of the polymer. Liu et al. (2013) described a low molecular weight EPS (10^4 Da) produced by *L. paracasei* isolated from Tibetan kefir grain. EPSs produced by *L. paracasei* strains from CIDCA collection had one or two fraction of high molecular weight being these strains the first described that produce an exopolysaccharides with a high molecular weight fraction. Since production of high molecular weight EPS normally affects the viscosity of fermented milk (Folkenberg, Dejmek, Skriver, & Ipsen, 2006; London et al., 2015) these strains could have a successful application improving texture of fermented dairy product.

3.2. *Fermented milk: flow curves analysis*

The fermentation of milk with lactobacilli leads to important changes in the textural characteristics of fermented product as consequence of the acidification and polysaccharide production. The microorganisms and/or the exopolysaccharides they produce may affect protein aggregation, interactions with milk constituents, and interactions between exopolysaccharides and the bacterial cell surface (Folkenberg, Dejmek, Skriver, Guldager, et al., 2006). The fermented milks were characterized by rotational viscometer measurement. Typical flow curves for milk gels obtained with different *Lactobacillus* species are shown in Fig. 1. All the fermented milk gels showed a pseudoplastic behavior with a hysteresis loop; however the magnitude of viscosity values and hysteresis loop depended on microorganism used for the fermentation of milk. Yovanoudi et al. (2013) studied milks fermented by two commercial starters containing EPS-producer lactobacilli. These authors also observed pseudoplasticity character and tixotropy in flow curves and they attributed it to the presence of EPS-water matrix which quickly broke down at high shear rates. The acid milk gels obtained by fermentation with all the strains of *L. paracasei* were the ones that presented the highest hysteresis loop. The increase in the loop area represents an additional structure of the EPS-protein network and in yoghurts, a relationship was found between hysteresis loop area and sensory ropiness (Folkenberg, Dejmek, Skriver, Guldager, et al., 2006).

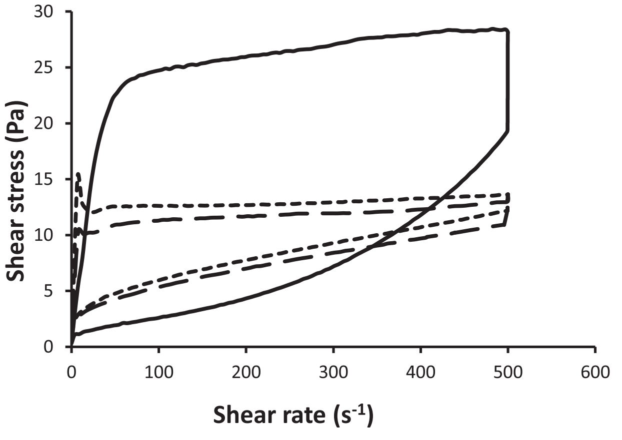

Fig. 1. Flow curves, evaluated at 25 °C, of milks fermented by *L. plantarum* (— —), *L. kefiranofaciens* (·····) and *L. paracasei* (——) strains.

The apparent viscosities values, at 300 s^{-1}, of fermented milks with all the *Lactobacillus* strains studied as well as the corresponded to chemically acidified milk are shown in Fig. 2. The apparent viscosity of chemically acidified milk was 23.1 ± 3.4 mPa s.

The highest viscosities values corresponded to the acid gels obtained by fermentation of milk with the *L. paracasei* strains studied. They were all higher ($p \leq 0.01$) than the corresponding value of chemically acidified milk. Likewise, fermented milks with strains of *L. plantarum* CIDCA 8323, CIDCA 8336, CIDCA 8312, CIDCA 83112 and CIDCA 8318 and *L. kefiranofaciens* CIDCA 83118 and CIDCA 83122 also exhibited apparent viscosity values which were higher ($p \leq 0.01$) than the value of chemically acidified milk but lower than the viscosities of fermented milk with all *L paracasei* strains ($p \leq 0.01$).

The experimental plots of the up flow curves satisfactory fitted the Ostwald de Waele model and the obtained consistency and flow indices are presented in Table 1.

The fermented milk flow index was among 0.03 to 0.23, indicating pseudoplastic behavior. The consistence index values (κ) were in concordance to viscosity values, being the milks fermented with *L. paracasei* strains the ones with the highest κ values.

In Fig. 3, the viscosity values are plotted as a function of polysaccharide concentration in each fermented product. Acid milk gels obtained by fermentation with studied *L. plantarum* and *L. kefiranofaciens* strains had viscosities values around 30 mPa s, which did not depend on EPS concentrations in agreement with results described by Gentès, St-Gelais, and Turgeon (2011). Viscosities of fermented milks with *L. paracasei* were higher compared to other fermented products that contain the same concentration of EPS, being the acid milk gels obtained by fermentation with *L. paracasei* CIDCA 83123 the one that presented the highest viscosity value. Nevertheless, EPS concentration is not the only parameter that may affect viscosity of the acid milk gel obtained, indicating that the effect of EPS could be more complex. Size and arrangement of these molecules significantly influence viscosity since the thickening effect of a polysaccharide in solution depends on its primary structure, degree of branching and flexibility of the backbone and its molecular weight (Ruas-Madiedo, Tuinier, Kanning, & Zoon, 2002).

Analyzing the distributions of molecular weight of the EPS produced by the *Lactobacillus* strains studied and the viscosities of the fermented milk it can be concluded that only the strains capable to producing high molecular weight polysaccharide

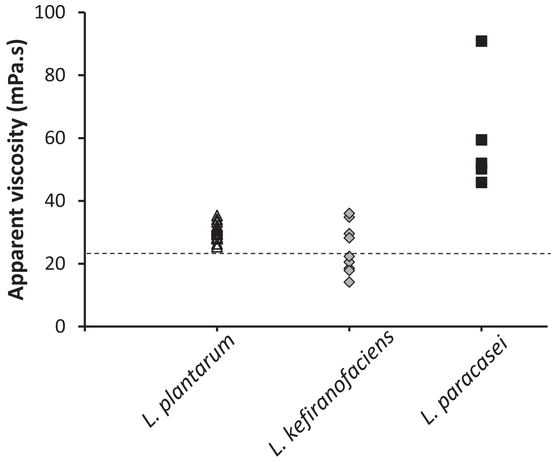

Fig. 2. Apparent viscosity (measured at 300 s^{-1}), of milks fermented with *L. plantarum* (△), *L. kefiranofaciens* (◇) and *L. paracasei* (■) strains. Dashed line showed value corresponded to milk acidified with glucono-δ-lactone (23.1 mPa s). Each symbol in the graph corresponds to average of three independent determinations.

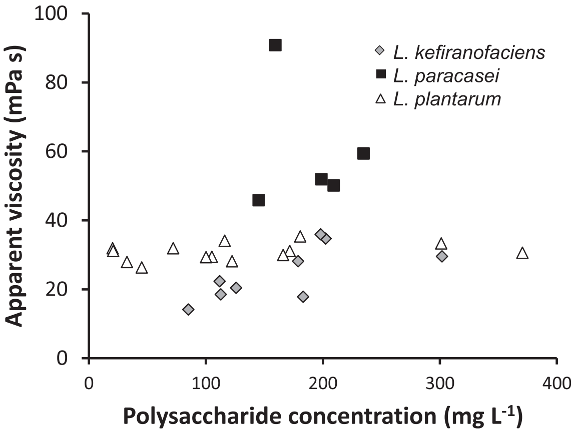

Fig. 3. Apparent viscosity (measured at 300 s^{-1}), of milks fermented with *L. plantarum* (△), *L. kefiranofaciens* (◈) and *L. paracasei* (■) strains, as a function of EPS concentration. The values corresponded to three independent measurements for each microorganism.

increased apparent viscosity values of fermented milks. *L. paracasei* strains that produce a high molecular weight EPS were the ones that produce acid milk gels with the highest values of apparent viscosity. The fermented milk with *L. paracasei* CIDCA 83123, which has the highest viscosity value among all the studied strains, contains the highest concentrations of EPS of molecular weight higher than 10^6 Da. The molecular weight of this polysaccharide can explain the high viscosity value in concordance to previous results (Petry et al. 2003; Tuinier et al., 2001). Likewise, it was described that EPS with a high molecular weight, a stiff chain and few branching improves apparent viscosity and whey retention of fermented milk (Gentès et al., 2011).

3.3. *Fermented milk: dynamic rheological measurements*

The contribution of EPS to the textural properties of food products depends on the properties of the EPS itself but also on the interactions with various components in food products (Girard & Schaffer-Lequart, 2007). For a better understanding of the role of EPS, produced *in situ*, on texture of fermented product, its dynamic rheological characteristics were determined. *L. paracasei* strains were selected for characterization of the resulting acid gel by small amplitude oscillatory shear measurements. These strains were selected because they produce polysaccharides with a high molecular weight fraction and the high viscosity of the fermented milks obtained with them.

Stress of 0.1 Pa, into the linear viscoelastic range, was chosen for frequency sweep assays of acid skim milk gels obtained by

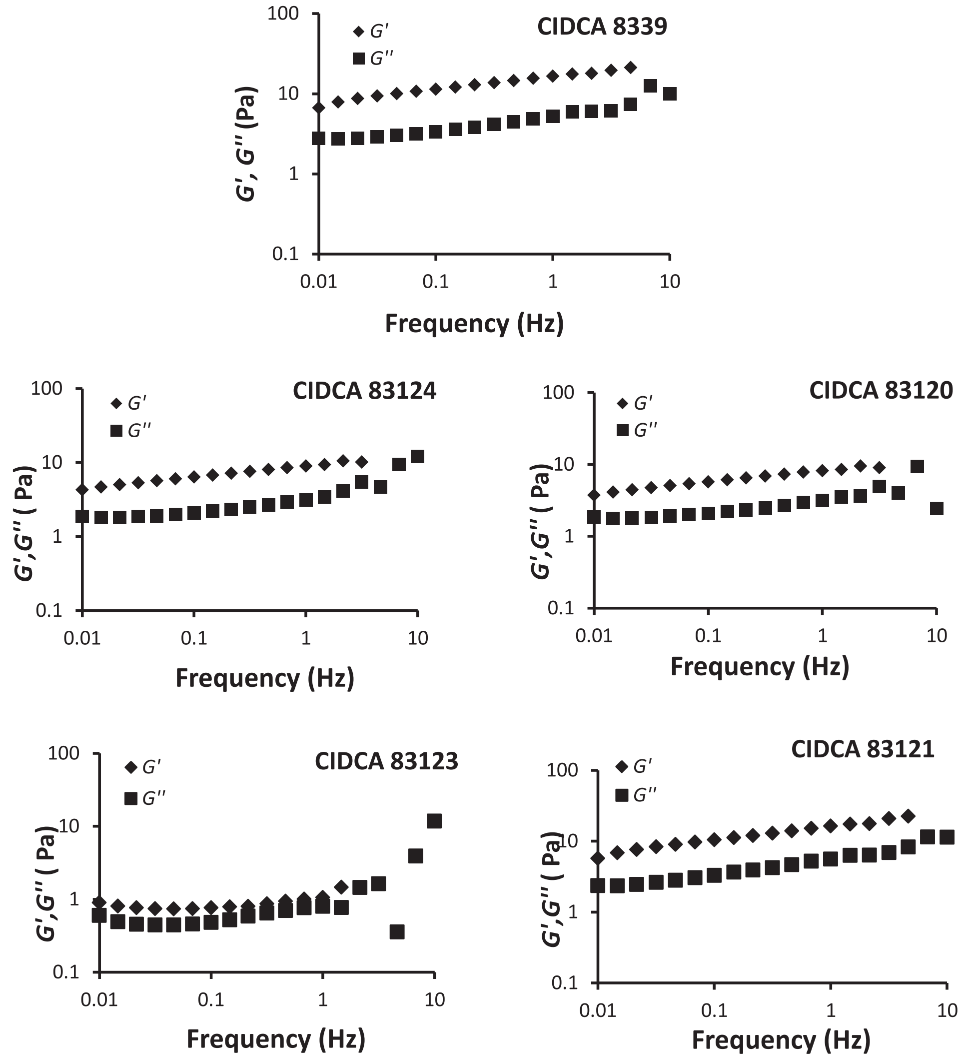

Fig. 4. Mechanical spectrum, evaluated at 0.1 Pa stress and 25 °C, corresponding to milk fermented with *L. paracasei* strains. Elastic modulus, G′ (♦) and viscous modulus, G″ (■).

fermentation of milk with *L. paracasei*. Mechanical spectra corresponding to fermented milks with *L. paracasei* CIDCA 8339, CIDCA 83120, CIDCA 83121 and CIDCA 83124 strains showed typical gel rheological behavior with the elastic modulus (G') higher than viscous modulus (G'') over the whole frequency range. On the contrary, acid gel obtained by milk fermentation with *L. paracasei* CIDCA 83123 presented a different behavior since G' and G'' had similar contribution indicating a fluid rheological behavior (Fig. 4).

Frequency of 1 Hz was selected to compare the elastic and viscous moduli values corresponding to the milks fermented by *L. paracasei* strains (Table 2). Acid milk gels obtained by fermentation with *L. paracasei* CIDCA 8339 and CIDCA 83121 had the highest elastic modulus values followed by the corresponding to fermented milks obtained with *L. paracasei* CIDCA 83120 and CIDCA 83124 ($p \leq 0.05$). As was previously mentioned, the fermented milk produced by strain CIDCA 83123 had a rheological behavior where the contribution of elastic modulus is low and equivalent to viscous modulus. The difference between the acid gels obtained by fermentation of milk with these strain can also be confirmed by the analysis of tan δ (Table 2) where the acid gel produced by *L. paracasei* CIDCA 83123 had a value equivalent to a viscous product and the other had values that correspond to week gel.

The higher values of the storage modulus (G') indicates interactions between the EPS and the milk protein network of the acid gel as was previously suggested (Ayala-Hernandez, Hassan, Goff, & Corredig, 2009; Gentès et al., 2011; Girard & Schaffer-Lequart, 2008). The polysaccharide tends to absorb to the protein surface leading to bridges between proteins that strengthen the gel structure leading to higher elastic characteristics (higher G' values). Stiffer gels could also derive by segregative interaction if EPS combines with water molecules and induce protein–protein interaction (Kleerebezem et al., 1999; Ruas-Madiedo et al., 2008).

Acid gels obtained by fermentation of milk with EPS producing-*Lactococcus lactis* subsp. *cremoris* resulted in a stiffer gel, with a higher viscous component (Kristo, Miao, & Corredig, 2011). Other studies have reported lower G' values (Doleyres, Schaub, & Lacroix, 2005; Hassan, Ipsen, Janzen, & Qvist, 2003) when milk was fermented with an EPS producing culture compared to control. Previous results demonstrated that neutral exopolysaccharide contributes to the viscosity but not to the elasticity of acid gel meanwhile negatively charged polysaccharide interact with the positively charged casein particles by electrostatic interactions, increasing G' (Pleijsier, de Bont, Vreeker, & Ledeboer, 2000). As a consequence different strains of lactic acid bacteria that produce either charged or neutral EPSs can be selected to tailor mouthfeel (Renard, van de Velde, & Visschers, 2006). A ropy character was clearly observed in the acid gel produced by strain *L. paracasei* CIDCA 83123 that was not observed in the fermented products with the other strains which further confirmed the different interaction of this particular EPS with the milk proteins and give an insight of the a different characteristic of the EPS produced.

Table 2
Storage modulus (G'), loss modulus (G'') and loss tangent (tan δ) at 1 Hz corresponding to milks fermented by *L. paracasei* strains. Each column represents the mean of three independent samples.

	Strain	G'/(Pa)	G''/(Pa)	tan (δ)
L. paracasei	CIDCA 8339	16.49 ± 0.40^{a}	5.22 ± 0.61^{a}	0.32 ± 0.12^{a}
	CIDCA 83120	8.60 ± 0.63^{b}	3.24 ± 0.11^{b}	$0.38 \pm 0.01^{a,b}$
	CIDCA 83121	16.54 ± 0.37^{a}	5.21 ± 0.54^{a}	0.32 ± 0.04^{a}
	CIDCA 83123	0.86 ± 0.27^{c}	0.68 ± 0.16^{c}	0.81 ± 0.07^{c}
	CIDCA 83124	5.36 ± 0.38^{d}	2.3 ± 0.14^{d}	0.49 ± 0.02^{b}

4. Conclusions

Exopolysaccharide produced during milk fermentation can improve the technological properties of fermented dairy products and potentially replace hydrocolloids. In this work we found that strain of *L. plantarum*, *L. kefiranofaciens* and *L. paracasei* are able to produce EPS *in situ* during fermentation of milk. The amount of EPS as well as the molecular weight distribution depended on the strain used. Rheological characterization of acid milk gels obtained indicates that viscosity did not depend on polysaccharide production but depend on molecular weight distribution of EPS.

Acid milk gels obtained by fermentation with *Lactobacillus* strains that produce low molecular weight EPS had low viscosities values although they produce high EPS concentrations. Viscosities of fermented milks with four strains of *L. paracasei* that produce EPS containing a high molecular weight fraction, are higher than fermented milk containing the same concentration of EPS and the fermented milk obtained with these lactobacilli presented mechanical spectra that characterize a gel structure. Acid milk gels obtained by fermentation with *L. paracasei* CIDCA 83123 presented the highest viscosity and mechanical spectra, was the one for a viscous product indicating different interaction of this particular EPS with the milk proteins. This is the first report that describes the production of high molecular weight EPS by *L. paracasei* strains and evaluates the rheological properties of acid milk gels obtained. This finding indicates that *L. paracasei* strains isolated from kefir grains are promising for the development of starters for milk fermentation with improved rheological properties.

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