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Full Length Research Paper

Influence of starter culture strains, pH adjustment and incubation temperature on exopolysaccharide production and viscosity in whey

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There are many functional characteristics of exopolysaccharides (EPS) produced by lactic acid bacteria in fermented dairy products. In this study, 2 EPS-producing strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and 2 *Streptococcus thermophilus* strains were isolated from commercial and rural yoghurts and they were inoculated into whey and their effects on EPS production and viscosity of whey were monitored. Two pH applications (adjusted pH and free pH) and 3 incubation temperature (42, 36 and 42/36ºC) were performed. pH of samples was significantly affected by starter culture strains, pH adjustment and incubation temperature ($P < 0.01$). Generally counts of *L. delbrueckii* ssp. *bulgaricus* were found higher than of *S. thermophilus* ($P < 0.05$). Viable cell counts were significantly higher, in pH-adjusted samples and 42ºC samples, than their relevants ($P < 0.01$). Higher concentrations of EPS were observed in pH-adjusted media than that in pH-free media ($P < 0.01$). Also 2-step (42/36ºC) and lower (36ºC) incubation temperature caused an increase in EPS production. Viscosity of samples greatly influenced from their EPS contents. The highest EPS concentration (676 mg/L) and viscosity (6.37 mPa.s) was determined in the sample that inoculated with *S. thermophilus* ST3 strain, incubated at 42/36ºC with pH-adjustment.

Key words: Lactic acid bacteria, exopolysaccharide, whey.

INTRODUCTION

Many functional characteristics of lactic acid bacteria (LAB) are responsible for their traditional and modern use in food production. One such characteristic that has recently generated much interest is polysaccharide production (Shihata and Shah, 2002). The LAB that produce exopolysaccharides (EPS) are often used to increase the viscosity of stirred fermented milks, such as yoghurt and to decrease the susceptibility to syneresis (Ruas-Madiedo et al., 2002a). Exopolysaccharides are polysaccharides external to the cell, either attached to cells or excreted as free polysaccharides (Cerning et al., 1992; Nakajima et al., 1990). Compositions and structures of EPSs are very heterogeneous and their effects on the product are also variable. Therefore many different terms are used to describe EPS produced by LAB. They have been described as mucoid, slime producing and ropy (Knoshaug et al., 2000). Also the molecular masses of the EPS from LAB very changeable and range from 10 kDa and over 200 kDa to >1,000 kDa (De Vuyst et al., 1998; Higashimura et al., 2000; Lemoine et al., 1997; Lo et al., 2007). In recent years several studies have focused on the rheological and textural properties of yoghurt made with EPS-producing strains (Bouzar et al., 1997; Hassan et al., 1996; Hess et al., 1997; Rawson and Marshall, 1997; Van Marle et al., 1999). Currently, EPS-producing LAB strains are used in yoghurt, sour cream and whipped toppings to improve their rheological properties, to prevent syneresis and to replace stabilizers (Wacher-Rodarte et al., 1993; Hess et al., 1997). Gassem et al. (1995) and Griffin et al. (1996) reported that polysaccharide - producing yoghurt bacteria were important determinants of yoghurt viscosity and texture. Industrial scale production of EPS from LAB is not very common since the yield of EPS biosynthesis is relatively low when compared with dextran-producing or gram-negative EPS-producing strains (De Vuyst and Degeest, 1999; Doleyres et al., 2005).

In most cases, the total amount of EPS synthesized

Table 1. Definition of starter cultures used in the experiment.

Starter culture code	Starter culture strain	Source
LB12	*Lactobacillus delbrueckii* ssp. *bulgaricus* LB12	Laboratory collection, rural yoghurt
LB18	*Lactobacillus delbrueckii* ssp. *bulgaricus* LB18	Laboratory collection, commercial yoghurt
ST3	*Streptococcus thermophilus* ST3	Laboratory collection, commercial yoghurt
ST7	*Streptococcus thermophilus* ST7	Laboratory collection, commercial yoghurt

does not exceed 1 g/L even under optimal growth conditions, although some higher amounts have been reported for *Lactobacillus returi* strains (4.1 and 4.8 g/L), *S. thermophilus* LY03 (1.5 g/L) and *L. sakei* 0 - 1 (1.4 g/L) (Lo et al., 2007). Nevertheless, the search for over producing strains and the better understanding of the mechanisms that regulate their synthesis could improve the yields to economically feasible levels.

The majority of EPS-producing microorganisms utilize carbohydrates as their energy source as well as their carbon source for EPS formation (Lo et al., 2007). Whey is an important by product of cheese manufacture and contains most of the lactose of cheese-milk. Sometimes whey was thrown away by cheese producers or used for feeding livestock. Currently, the major usage of whey and whey permeate are in manufacturing dried whey powder and refined lactose. These usages, however, are often aimed at keeping the surplus whey out of sewers rather than producing a highly desirable product. The ultimate goal for the dairy industry should be to turn whey lactose into a product with high value. Thus, by the production of EPS, whey will be utilized for the production of valuable products. EPS produced by LAB using whey can be utilized in the dairy products and bakery products such as cakes. Coherent with this premise, in a study conducted our laboratories, partially purified and concentrated EPS were successfully used to reduce oil/fat requirement in cake production.

The aim of this study was to investigate the effect of LAB strains, incubation temperature and pH development on EPS production and viscosity in whey. In order to do this, 2 EPS-producing strains of *L. delbrueckii* ssp. *bulgaricus* and 2 *S. thermophilus* strains were isolated from commercial and rural yoghurts and they were inoculated into whey and their effects on EPS production and thickening efficiency were monitored.

MATERIALS AND METHODS

Bacterial strains and propagation media

2 EPS-producing *L. delbrueckii* ssp. *bulgaricus* strains and 2 EPS-producing *S. thermophilus* strains previously isolated from commercial yoghurts were used in whey fermentation.

Stock cultures were kept frozen in 12% (wt/vol) skim milk and 25% (wtv) glycerol at -20°C. For inoculum preparation, 1% of the frozen *L. delbrueckii* spp. *bulgaricus* strains and 1% *S. thermophilus* strains were propagated separately in 10% (wt/vol) skim milk medium at 42°C for 5 h. Definition of starter cultures was given in Table 1.

Whey properties and fermentation conditions

Whey was obtained from cheese production without starter culture addition. Fat of whey was removed by centrifugal cream separation and then whey was sterilized at 121°C for 15 min. After cooling to 45°C, whey was filtered using sterilized glass wool and filter flasks to remove the heat-formed precipitate. Then whey content was enriched by the addition of 5% (v/v) sterilized skim milk. Composition of fermentation media was as follows: total solids 5.54 - 5.63%; crude protein 0.37 - 0.41%; fat < 0.1%; pH 6.42 - 6.44 and titration acidity 0.15 - 0.17%. Whey (200 mL) was dispensed into 250 ml sterilized glass jars with screw cap. Whey samples were divided into 2 groups. pH adjustment was applied in the first group and the pH was maintained at the set point (pH 5.5) by the addition of 5 N NaOH. Maximum NaOH addition was determined as 2.25 ml/200 mL per whey sample. Thus, at the end of fermentation, volume of the other samples was made up to 202.25 mL with water. No pH adjustment was applied in the other group and incubation was performed with a free pH. However, volume of these samples was also adjusted. 3 different incubation temperatures were used for each group. The first batch was incubated at 42°C, the second batch at 36°C and the third batch at 42°C for 2 h and then at 36°C. Whey samples were inoculated with the starter cultures indicated above at the ratio of 1%. Fermentation was performed in static condition; however fermentation media were agitated sometimes by shaking the jars with hand. Thus, the experiment was conducted using 4 starter cultures, 2 pH applications and 3 incubation temperatures. Incubation was performed for 18 h and study was performed in triplicate.

Chemical analyses

The total solids were determined by oven-drying method (AOAC, 1990); pH of the samples was measured by pH meter (Hanna Instruments, Cluj-Napoca, Romania); total nitrogen was determined by micro-Kjeldahl procedure (Simsek Laborteknik, Ankara, Turkey) (Case et al., 1985) and crude protein was calculated by % total N × factor of 6.38. Titration acidity and fat content were also carried out according to the methods described by Case et al. (1985).

Enumeration of lactic acid bacteria

MRS Agar (de Man, Rogosa, Sharpe; Oxoid Ltd. Hampshire, England) was used for counting *L. delbrueckii* ssp. *bulgaricus*. 5% lactose added M17 Agar (Oxoid Ltd. Hampshire, England) was used for enumeration of *S. thermophilus*. Inoculated plates were incubated at 42°C for 72 h and then formed colonies were counted. Counts of bacteria were stated in text as log number.

EPS purification and quantification

EPS purification was performed by the method given by Amatayakul et al. (2006) with some modifications. For this reason, proteins in 50 mL of whey sample (1:1; whey : deionized water) (MES Ltd. Sti., Ankara, Turkey) were precipitated by adding 4 mL of 20% (wt/vol) TCA (Merck, Darmstadt, Germany). They were kept at 12°C for 3 h to accomplish the precipitation reaction. Precipitated proteins were separated by centrifugation (Hettich, Tuttlingen, Germany) at 3500 x *g* for 30 min at 4°C. The pH of supernatant was adjusted to 6.80 with 40% (wt/vol) NaOH followed by boiling the supernatant at 100°C for 30 min to denature whey proteins. Denatured whey proteins were separated by centrifugation at 3500 x *g* for 30 min at 4°C. Equal volume of cold absolute ethanol was mixed with the supernatant to precipitate the carbohydrate from the supernatant. The precipitation was carried out overnight at 4°C and the precipitate was separated by centrifugation at 3500 x *g* for 30 min at 4°C. The resultant carbohydrate pellet was completely dissolved by adding 10 mL of deionized pure water and the resultant suspension was subjected to sonication in a ultrasonic water bath (Bandelin, Berlin, Germany) for 1 h at room temperature. After that, the solution was dialysed at 4°C in a dialysis membrane tube with molecular weight cut-off 12 kDa (Sigma-Aldrich, St Louis, MO, USA) against distilled water for 3 days on a rotating-shaker incubator (Heidolph, Schwabach, Germany). Water : sample ratio was 20:1. Water was changed 4 times in a day. After this time, the samples were freeze-dried (Edwards, Crowley Susex, England), weighed and then EPS values were calculated (Gassem et al., 1997). Nitrogen contents of these samples were also determined and subtracted from these values. Thus, the final EPS values were expressed as mg /L of whey.

Determination of viscosity

Viscosity measurements were performed on 16 mL samples by using Brookfield digital rheometer model DV III Ultra (Brookfield Engineering Laboratories Inc., Stoughton, Massachusetts, USA). The rheometer was equipped with ultra low adapter (Brookfield Engineering Laboratories Inc), which rotated in a sample-containing chamber connected to a temperature-controlled cryostat water bath (Brookfield Engineering Laboratories Inc). The rheometer was controlled with the Brookfield Rheocalc V3.1-1 software (Brookfield Engineering Laboratories Inc). Apparent viscosity measurements in samples were performed at 25°C. Spindle speeds of 0 - 240 rpm with 5 rpm interval were applied for 30 s. However, for the comparison of the samples between the different applications, a spindle speed of 200 rpm was chosen. The torque was between 14.1 and 23.7% and shear rate was 244.60 sec^{-1} at this rotation. All viscosity measurements were expressed in mPa.s, performed in duplicate and averaged.

Statistical analysis

The data were analyzed using 2-way ANOVA at 95% confidence level with SAS (2005). The means of data were expressed with standard deviation and compared with Duncan test.

RESULTS AND DISCUSSION

Growth of lactic acid bacteria in whey and change of pH

Expectedly, pH of pH-adjusted whey samples was determined as almost stable (pH 5.5) at the end of fermentation, however, pH values were significantly (P < 0.01) decreased in the samples fermented without pH adjustment. pH values of samples fermented at 42ºC were found significantly (P < 0.01) lower than that of others (Table 2). As known, optimal growth temperature of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are over 40ºC (Ucuncu, 2005). Thus, decrease in the fermentation temperature had caused lower starter activity. The highest glycolytic capacity was determined in LB12 sample. Viable cell counts (*L. delbrueckii* sub sp. *bulgaricus*) were also higher in this sample than the other samples. pH and buffering capacity of fermentation media can change depending on their composition and especially protein concentration (Amatayakul et al., 2005). However, composition of all fermentation media was the same in this study.

There was significant effect of pH adjustment (P < 0.05), fermentation temperature (P < 0.01) and starter culture (P < 0.05) factors on the growth of starter bacteria. NaOH addition into fermentation medium to control of pH generally enhanced the growth of starters used. While mean viable count was 7.09 log cfu/g for pH-adjusted media, it was 6.75 log cfu/g for pH-free media. The highest counts of bacteria were observed at 42ºC when compared with other incubation temperatures. On the other hand, incubation at 42/36ºC had positive effect on the growth of bacteria. The number of viable cells of the *L. delbrueckii* ssp. *bulgaricus* LB12 strain was signifycantly (P < 0.05) higher than that of other strains. The lowest viable cell numbers were determined in ST3 and ST7 samples (Table 2). It was reported that viable cell counts can change in yoghurt depending on the strain used (Amatayakul et al., 2005). In general, *L. delbrueckii* ssp. *bulgaricus* counts were found lower in this study than determined in yoghurt (Bouzar et al., 1997) even at 42ºC. Also viable counts of *S. thermophilus* were generally found lower than that of results reported by De Vuyst et al. (2003) and Vaningelgem et al. (2004). However, milk or enriched milk medium was used in these researches. Probably, these media are more suitable than whey for the bacteria. Despite of obtaining slightly lower counts of bacteria in this study, composition of whey was reported as sufficiently complex for growth of lactic acid bacteria (Grobben et al., 1998).

EPS concentration and viscosity of whey samples

Higher concentrations of EPS for all incubation temperatures and for all strains were observed in pH-adjusted media than pH-free media (P < 0.01). While mean EPS production for pH-adjusted media was 468 ± 124 mg/L, this value was 316 ± 96 mg/L for pH-free media (Table 3). pH is one of the most important factors affecting metabolic activity of starter cultures in fermented products (Degeest et al., 2001). Also the factor of incubation temperature had significant effects (P < 0.01) on EPS production and the highest amount of EPS was determined

Table 2. pH change and viable cell counts in fermented whey samples*.

Parameter	Starter culture code	Adjusted pH (5.5)				Free pH				Mean ± SD
		Incubation temperature (ºC)			Mean ± SD	Incubation temperature (ºC)			Mean ± SD	
		42	36	42/36		42	36	42/36		
pH	LB12	5.50 ± 0.02	5.49 ± 0.03	5.50 ± 0.04	5.49 ± 0.03	4.04 ± 0.09	4.82 ± 0.16	4.23 ± 0.06	4.36 ± 0.36	4.93 ± 0.63 ^{c}
	LB18	5.49 ± 0.03	5.50 ± 0.01	5.51 ± 0.03	5.50 ± 0.02	4.01 ± 0.14	5.06 ± 0.09	4.35 ± 0.07	4.47 ± 0.47	4.99 ± 0.62 ^{ab}
	ST3	5.52 ± 0.02	5.51 ± 0.03	5.49 ± 0.03	5.50 ± 0.02	4.22 ± 0.07	4.96 ± 0.10	4.35 ± 0.11	4.51 ± 0.35	5.01 ± 0.56 ^{a}
	ST7	5.49 ± 0.02	5.48 ± 0.01	5.50 ± 0.03	5.49 ± 0.02	4.05 ± 0.08	4.87 ± 0.08	4.24 ± 0.07	4.39 ± 0.37	4.94 ± 0.62 ^{bc}
	Mean± SD	5.50 ± 0.02	5.49 ± 0.02	5.50 ± 0.03	5.50 ± 0.02 ^{A}	4.08 ± 0.12	4.93 ± 0.13	4.29 ± 0.09	4.43 ± 0.38 ^{B}	
	Mean ± SD depending on incubation temperature 42 ºC = 4.79 ± 0.72 ^{c} 36 ºC = 5.21± 0.30 ^{a} 42/36 ºC = 4.89±0.61 ^{b}									
Viable cell counts (log cfu/g)	LB12	6.98 ± 0.73	7.42 ± 0.54	7.17 ± 0.46	7.19 ± 0.55	8.08 ± 0.61	6.72 ± 0.58	6.92 ± 0.20	7.24 ± 0.77	7.21 ± 0.65 ^{a}
	LB18	7.16 ± 0.39	7.22 ± 0.68	7.15 ± 0.50	7.18 ± 0.46	7.00 ± 0.21	6.20 ± 0.23	6.63 ± 0.09	6.61 ± 0.38	6.89 ± 0.50 ^{ab}
	ST3	7.06 ± 0.37	7.05 ± 0.70	6.84 ± 0.44	6.98 ± 0.46	6.82 ± 0.29	6.04 ± 0.76	6.82 ± 0.54	6.56 ± 0.62	6.77 ± 0.58 ^{b}
	ST7	7.36 ± 0.48	6.42 ± 0.37	7.21 ± 0.56	6.99 ± 0.60	7.54 ± 0.65	5.89 ± 0.27	6.33 ± 0.62	6.59 ± 0.87	6.79 ± 0.76 ^{b}
	Mean ± SD	7.14 ± 0.46	7.03 ± 0.63	7.09 ± 0.45	7.09 ± 0.52 ^{A}	7.36 ± 0.65	6.21 ± 0.54	6.67 ± 0.43	6.75 ± 0.71 ^{B}	
	Mean ± SD depending on incubation temperature 42 ºC = 7.25 ± 0.56 ^{a} 36 ºC = 6.62 ± 0.71 ^{b} 42/36 ºC = 6.88 ± 0.48 ^{b}									

* Mean values ± standard deviation of three trials.
^{abc}letters indicate differences ($P < 0.05$) between mean values of cultures
^{A,B} letters indicate differences ($P < 0.05$) between mean values of pH adjustment.
^{abc} letters indicate differences ($P < 0.05$) between mean values of incubation temperature.

in the samples incubated at 42/36ºC. It seems that, incubation of bacteria at 42ºC both increased viable cell and stimulated acid production but retarded EPS production. Coherent with this premise, it can be seen from Table 2 that the highest mean cell counts and the lowest mean pH value has been determined in the samples fermented at 42ºC. Amount and character of EPS can be affected by incubation conditions such as pH, incubation temperature and period and composition of media (Garti and Reichman, 1993; Fajardo-Lira et al., 1997; Ruas-Madiedo et al., 2002b).

ST3 sample has possessed the highest mean EPS concentration. EPS production of this sample has also reached to 676 mg/L when pH was adjusted (5.5) and 2-step temperature (42/36ºC) was applied. In a study, synthesis of EPS by *L. delbrueckii* ssp. *bulgaricus* RR strain in semi-defined medium varied greatly depending on incubation temperature and pH of medium; the highest EPS concentration was obtained at 38ºC and pH 5.0 (Kimmel et al., 1998). The quantities of EPS produced in milk by different species and strains vary considerably. The EPS quantities produced in milk by different strains of *Lactococcus lactis* sub sp. *cremoris* range from 80 to 600 mg/L and even lower yields (25 mg/L) have been reported (Cerning, 1995).

In the previous studies, the amounts of EPS reported range from 50 to 350 mg/L for *S. thermophilus* (Cerning et al., 1988; Doco et al., 1990), from 60 to 150 mg/L for *L. bulgaricus* (Cerning et al., 1986; Garcia-Garabay and Marshall, 1991). However, higher EPS production has been reported for *S. thermophilus* by Degeest and De Vuyst (1999) and Zisu and Shah (2003) and for *L. delbrueckii* ssp. *bulgaricus* by Grobben et al. (1998). In a study, a very high EPS concentration of 2.3 g/L was ob-tained during pH-controlled batch cultures of *Lactobacillus rhamnosus* RW-9595M in 8% whey permeate medium supplemented with some minerals and yeast extract (Bergmaier et al., 2003). Similar yield (2.2 g/L) was obtained by Doleyres et al. (2005) using this strain. In the studies stated above, generally various enrichment agents or additives has been practiced in order to improve EPS production in milk or milk based media. However, the aim of this study was to determine the productivity of yoghurt cultures in whey medium without enrichment with other carbon sources and keep the cost of product

Table 3. Exopolysaccharide concentration (mg/L) and viscosity (mPa.s) of whey samples*.

Parameter	Starter culture code	Adjusted pH (5.5)				Free pH				Mean ± SD
		Incubation temperature (ºC)			Mean ± SD	Incubation temperature (ºC)			Mean ± SD	
		42	36	42/36		42	36	42/36		
EPS (mg/L)	LB12	311 ± 27.6	436 ± 44.5	457 ± 44.8	401 ± 76	203 ± 27.0	259 ± 25.3	296 ± 23.8	253 ± 46	327 ± 97 ^{c}
	LB18	261 ± 21.7	479 ± 56.5	619 ± 41.0	453 ± 160	183 ± 16.5	378 ± 35.7	410 ± 41.1	324 ± 110	388 ± 149 ^{b}
	ST3	454 ± 25.54	633 ± 34.5	676 ± 6.5	588 ± 104	333 ± 50.4	438 ± 45.5	482 ± 61.1	418 ± 80	503 ± 125 ^{a}
	ST7	392 ± 18.44	431 ± 50.1	464 ± 43.0	429 ± 46	283 ± 43.6	245 ± 19.6	283 ± 41.0	270 ± 36	350 ± 91 ^{c}
	Mean± SD	355 ± 79	494 ± 94	554 ± 104	468 ± 124 ^{A}	250 ± 70	330 ± 89	368 ± 93	316 ± 96 ^{B}	
	Mean ± SD depending on incubation temperature	42 ºC = 302 ± 90 ^{c}	36 ºC = 412 ± 123 ^{b}	42/36 ºC = 461 ± 136 ^{a}						
Viscosity (mPa.s)	LB12	3.50 ± 0.03	4.33 ± 0.08	4.42 ± 0.15	4.08 ± 0.45	3.07 ± 0.09	4.00 ± 0.08	3.69 ± 0.17	3.59 ± 0.42	3.83 ± 0.49 ^{c}
	LB18	3.54 ± 0.03	5.10 ± 0.12	6.16 ± 0.52	4.93 ± 1.17	3.21 ± 0.13	4.37 ± 0.17	5.00 ± 0.14	4.19 ± 0.79	4.56 ± 1.04 ^{b}
	ST3	4.70 ± 0.07	5.95 ± 0.15	6.37 ± 0.28	5.67 ± 0.77	3.81 ± 0.08	5.16 ± 0.29	5.14 ± 0.20	4.70 ± 0.69	5.19 ± 0.86 ^{a}
	ST7	3.82 ± 0.11	4.11 ± 0.11	4.19 ± 0.10	4.04 ± 0.19	3.40 ± 0.07	3.63 ± 0.16	3.61 ± 0.20	3.55 ± 0.17	3.79 ± 0.31 ^{c}
	Mean ± SD	3.89 ± 0.51	4.87 ± 0.76	5.28 ± 1.06	4.68 ± 0.98 ^{A}	3.37 ± 0.30	4.29 ± 0.61	4.36 ± 0.75	4.01 ± 0.73 ^{B}	
	Mean ± SD depending on incubation temperature	42 ºC = 3.63 ± 0.48 ^{c}	36 ºC = 4.58 ± 0.74 ^{b}	42/36ºC = 4.82 ± 1.01 ^{a}						

* Mean values ± standard deviation of 3 trials.
^{abc} letters indicate differences (*P*<0.05) between mean values of cultures
^{A,B} letters indicate differences (*P*<0.05) between mean values of pH adjustment.
^{abc} letters indicate differences (*P*<0.05) between mean values of incubation temperature.

lower. Therefore, only skim milk was added into whey at the ratio of 5%. In the preliminary study, growth of bacteria was found insufficient in whey. However, sufficient cell growth was obtained when the whey enriched with 5% milk addition. Thus, both starter growth and EPS production was found satisfactory when the media enriched with milk.

Viscosity of the samples was significantly affected by the factors of starter culture (P < 0.01), pH adjustment (P < 0.01) and incubation temperature (P < 0.01). The highest mean viscosity was determined in ST3 sample (5.67mPa.s) and this followed by LB18 sample (4.93mPa.s). There was a clear effect of pH adjustment on the viscosity of whey samples. pH-adjusted media possessed higher viscosity values than free pH applied samples. Viscosity values were also influenced by incubation temperature and the highest mean viscosity was determined in the samples incubated at 42/36ºC (4.82 mPa.s). Viscosity of fermented whey samples was strongly affected by their EPS contents. Correlation coefficient between the EPS concentration and the viscosity was very significant (r^2 = 0.905; P < 0.01). Although some authors suppose a direct correlation between viscosity of stirred yoghurts and EPS concentration, no clear relation has been demonstrated (Dupont et al., 2000; Sebastiani and Zelger, 1998; Van Marle and Zoon, 1995; Wacher-Rodarte et al., 1993), except that if a given strain produces more EPS the viscosity of the fermented milk will increase (Sebastiani and Zelger, 1998). Other factors, such as the molecular characteristics of the EPS will play a part (Ruas-Madiedo et al., 2002b). Most of the viscosity measurements in the literature were carried out using yoghurt (Hassan et al., 1996; Hess et al., 1997; Rawson and Marshall, 1997; Van Marle et al., 1999). As it is known, rheology of yoghurt is very different from whey due to its gel structure and composition. Protein content, heat treatment and the presence of milk fat, thickening agents (stabilizers) and bacterial exopolysaccharides are factors with a direct effect on the structure of the protein matrix of yoghurt (Lucey, 2004; Herrero and Requena, 2006). Whereas, material of this study was whey and it is a very simple medium when compared to yoghurt. Thus, it can be said that viscosities of whey samples were greatly formed depending on their EPS contents.

At present EPS yields in LAB fermentations are still too low to consider them as an alternative to polysaccharides from animal and plant sources. Nevertheless, the search for over producing strains and the better understanding of the mechanisms that regulate their synthesis could improve the yields to economically feasible levels.

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