Exopolysaccharide Production and Texture-Promoting Abilities of Mixed-Strain Starter Cultures in Yogurt Production

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ABSTRACT

An exopolysaccharide-producing strain of *Lactobacillus delbrueckii* ssp. *bulgaricus* (CNRZ 1187) and two colonial white and pink variants were grown in skim milk in association with a strain of *Streptococcus thermophilus* (CNRZ 389) that did not produce exopolysaccharide to assess the effects of associative growth on exopolysaccharide formation. After 10 h of fermentation, viscosity (380 mPa.s) was highest with the parental strain CNRZ 1187 in the mixed-strain starter, the lowest (220 mPa.s) with the mixed-strain starter containing the white variant and *S. thermophilus* CNRZ 389, and intermediate (290 mPa.s) with the mixed-strain starter containing the pink variant and *S. thermophilus* CNRZ 389. Viscosities of the milks with exopolysaccharide-producing strains decreased after mechanical stirring, but values remained higher (170 to 230 mPa.s) than those obtained with nonproducing strains (70 mPa.s). The mixed-strain starter composed of the white colonies of *L. delbrueckii* spp. *bulgaricus* and *S. thermophilus* produced the highest amount of exopolysaccharide (240 mg/L), and the mixed-strain starter containing the parental strain CNRZ 1187 produced the lowest amount (110 mg/L) of exopolysaccharide. The exopolysaccharide production from the mixed-strain starter containing the white and pink *L. delbrueckii* ssp. *bulgaricus* colonies began earlier and was higher after 3 h of fermentation than that from the starter containing the parental *L. delbrueckii* ssp. *bulgaricus* strain. Production of exopolysaccharide from the latter approached the maximum in the beginning of the stationary growth phase; production from the mixed-strain starter with the pink and white variants continued during the early stationary growth phase. The exopolysaccharide contained mainly galactose with small amounts of glucose and rhamnose. The monosaccharide composition of the exopolysaccharide changed during the exponential growth phase (6 h) and remained stable thereafter.

(**Key words**: yogurt, exopolysaccharide, mixed-strain starter culture)

Abbreviation key: **EPS** = exopolysaccharide, **P** = pink, **W** = white.

INTRODUCTION

The type and character of the starter organisms that are used in the production of fermented milks are two of the most important factors determining the overall quality of the final product. The essential criteria for starter selection include acidification, aroma, flavor, stability, and texture. Manufacturers can improve the body and texture of the product by manipulation of the composition of the yogurt mix, heat treatment of the mix prior to incubation, starter culture selection, incubation conditions, and addition of stabilizers. However, stabilizers can adversely affect the true yogurt taste, aroma, and mouthfeel. An alternative way to improve yogurt texture and stability is the use of bacteria that produce exopolysaccharides (**EPS**).

A considerable amount of information has become available in the last 10 yr demonstrating the increasing interest in these EPS-producing bacteria for making fermented milks (6, 7, 17, 20, 22). The quantities of EPS and the viscosity of milk containing different species of lactic acid bacteria in pure strain cultures vary considerably. The amounts of EPS range from 50 to 350 mg/L for *Streptococcus thermophilus* and from 60 to 150 mg/L for *Lactobacillus delbrueckii* ssp. *bulgaricus* (12, 17, 33). Viscosities of skim milk fermented with *S. thermophilus* ranged from 50 to 250 mPa.s, and viscosities of milk fermented with different strains of *L. delbrueckii* ssp. *bulgaricus* ranged from 100 to 220 mPa.s. (6, 7, 8). The EPS have high relative molecular masses (0.5×10^6) for *L. delbrueckii* ssp. *bulgaricus* (6) and *S. thermophilus* (1×10^6) (14). The ability to produce EPS is not a stable characteristic, as has been reported by numerous investigators, (7, 17), who observed that certain strains of lactic acid bacteria gradually lost the

property to promote texture. This spontaneous loss of the EPS-producing ability has been related to plasmid-encoded genes in mesophilic strains (24, 34), but the mechanism for regulation of this phenotype in thermophilic lactic acid bacteria is unclear.

The EPS from lactic acid bacteria are neutral heteropolysaccharides containing galactose, glucose, and rhamnose in varying proportions (6, 7, 11, 18, 20, 27, 30). In addition, N-acetyl galactosamine was identified in EPS from *S. thermophilus* (12), and N-acetyl glucosamine was found in EPS from *L. helveticus* (35). The structures of the repeating units of EPS produced by some lactic acid bacteria, including *L. delbrueckii* ssp. *bulgaricus* (20) and *S. thermophilus* (14), have been elucidated previously. Some of the EPS have been analyzed for their intrinsic viscosity and show remarkable thickening properties (8, 13).

During their associative growth, *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* stimulate each other in acid development, proteolysis (26), aroma production (23), and growth (1). However, results cannot be generalized because they are often strain-dependent (36). Very little is known about EPS production and texture-promoting abilities in mixed-strain cultures, except for results from the studies on the microstructure of ropy yogurt by Bottazzi and Bianchi (4), Schellhaass and Morris (28), and Teggatz and Morris (31). Those researchers showed that EPS is attached to the bacterial cell surface and also interacts with the caseins. The interaction between EPS and bacteria is disrupted when yogurt is sheared, but, after the EPS is separated from the cell surface, EPS continues to interact with the caseins and influences apparent viscosity (31).

In our previous study (5), we showed that a single pure strain of *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 has a heterogeneous cell constitution and that the colonial variability is correlated to EPS production and the ability to impart texture. Knowledge is almost completely lacking about the influence of associative growth of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* on both EPS production and sugar composition of EPS and texture. Therefore, this research was undertaken to study the associative growth of the EPS-producing *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 and its two colonial variants with a non-EPS-producing *S. thermophilus*. Milks fermented with these mixed-strain cultures presented different textural characteristics and varying EPS yields. In addition, we report that the EPS is produced more rapidly in the mixed-strain cultures and that the sugar composition is different from that obtained with single pure cultures.

MATERIALS AND METHODS

Strains

The EPS-producing (CNRZ 1187) and nonproducing (CNRZ 398) strains of *L. delbrueckii* ssp. *bulgaricus* and a nonproducing strain of *S. thermophilus* (CNRZ 389) were obtained from the freeze-dried culture collection of the Institut National de la Recherche Agronomique (Jouy-en-Josas, France). Two EPS-producing pink (**P**) and white (**W**) colonial variants, isolated from *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 as previously described, were used also (5). These variants, reflecting heterogeneity in formation of EPS, were isolated from the surface of a solid semisynthetic medium containing skim milk, sucrose, yeast extract, and ruthenium red (80 mg/L) (16).

The mixed-strain starters used were three combinations of the nonproducing *S. thermophilus* CNRZ 389 with the parental *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 and the W and P variants. These starters are referred to as 1) St/Lb 1187 culture, 2) St/Lb W culture, and 3) St/Lb P culture.

Culture Conditions

The culture conditions used were reported previously (5), except that samples were analyzed after 1.5, 3, 4.5, 6, 7.5, 9, 10.5, and 24 h of incubation at 42°C. The fermented milks were made from sterilized (110°C for 10 min), reconstituted NDM (10%, wt/vol). For single cultures with nonproducing strains, milk was inoculated with 1% overnight precultures. For the mixed-strain cultures, the strains were propagated separately, and the final 1% inoculum contained equal proportions of the single strains. After incubation, the cell numbers were determined microscopically as previously described (5), according to the method of Breed (32); the resulting values were expressed as direct microscopic counts.

Chemically acidified gels were made; 2% (wt/vol) glucono-δ-lactone was added to the milk to compare the viscosities of the gels derived from acidification alone and of the fermented milks containing EPS. This experiment was conducted at 25°C because chemical acidification simulates microbial acidification better at this temperature than at 42°C. The pH values of the two gel types, microbially acidified gel and chemically acidified gel, were measured with a pH electrode (Ingold, Urdof, Switzerland).

Viscosity

Rotational viscosity measurements were made with a coaxial cylinder viscosimeter (Rotovisco RV2;

Haake, Karlsruhe, Germany) at a steady shear rate of 173/s, using an MK 50 rotor assembly and an NV sensor system operating at 25°C. Viscosity was expressed as millipascals per second.

Viscosities were measured before and after stirring to test the resistance of the yogurt to mechanical handling. For that, yogurts were stirred on a magnetic stirrer for 1 min, and viscosity was measured thereafter.

Sugar Analysis

The concentrations (grams per liter) of lactose, glucose, and galactose in the culture supernatant were determined by HPLC after the proteins were precipitated in the sample with TCA at 40% as previously described (5).

EPS Isolation and Characterization

After hydrolysis of the caseins (proteinases from *Streptomyces griseus*; Boehringer, Mannheim, Germany), the EPS were isolated by ethanol precipitation as previously described (7). Total sugar concentration was determined by the phenol-sulfuric method (10) using glucose as a standard. The results are expressed in milligrams of glucose per liter.

Monosaccharide Composition

Quantitative analysis by gas-liquid chromatography of the sugars according to the method of Blakeney et al. (3) was described previously (5).

RESULTS

Rate of Growth

In the three milks fermented with mixed-strain starter cultures, the maximum population (1.3×10^8 to 2.4×10^9 DMC/ml) was attained after 6 h of incubation. The proportions of the two organisms (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) were slightly different in the three yogurts and did not vary substantially during the fermentation cycle.

Acid Development

There were few differences in acid development among the milks fermented with the mixed-strain cultures. The yogurts reached high acidities; pH values were as low as 3.8 after 24 h of fermentation. Acidification of milk induced by glucono-δ-lactone was
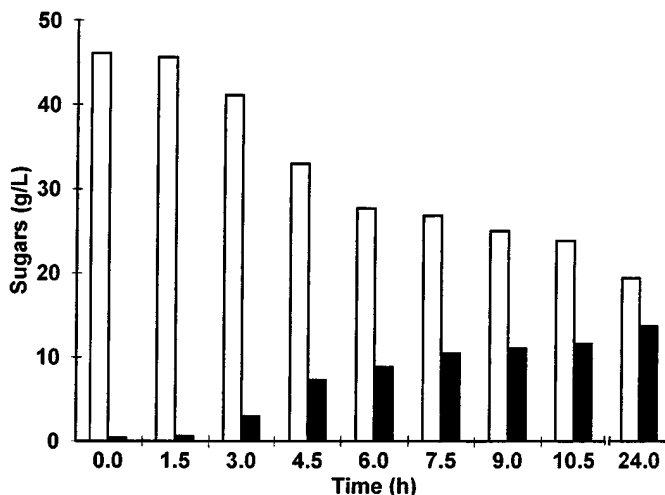
Figure 1. Residual lactose (□) and galactose (■) concentrations during a mixed-strain culture fermentation in reconstituted NDM (10%, wt/vol) at 42°C by the parental exopolysaccharide (EPS)-producing strain *Lactobacillus delbrueckii* ssp. *bulgaricus* CNRZ 1187 with non-EPS-producing *Streptococcus thermophilus* CNRZ 389 (St/Lb 1187). The values are the means of two measurements, and the maximum deviation between duplicate results is 8%.

faster than that obtained with bacterial fermentation with the lowest pH of 4.0 after 24 h.

Sugar Analysis

Few differences had been observed previously in lactose utilization among the single-strain cultures of *L. delbrueckii* ssp. *bulgaricus* (5). Therefore, sugar analysis was carried out on the St/Lb 1187 culture only. The initial lactose concentration of 46.1 g/L decreased progressively and reached 19.4 g/L after 24 h of fermentation (Figure 1). No free glucose was detected during the fermentation cycle, and galactose concentrations increased progressively from 3 h to 24 h of incubation, reaching 13.7 g/L at the end of the fermentation cycle.

Viscosity

The milks fermented with the St/Lb 1187 and St/Lb W cultures showed progressively increasing viscosities (Figure 2); maximum values (390 and 250 mPa.s) were obtained after 24 h of fermentation. The St/Lb P culture showed a similar increase up to 7 h 30 min of incubation with a viscosity of 300 mPa.s, but viscosity then decreased and was only 200 mPa.s after 24 h of fermentation. The visual appearances of the fermented milks were the same; the gels were smooth and without syneresis. In comparison, the viscosities of milks fermented with the nonproducing single-strain cultures (Figure 2) increased only

TABLE 1. pH values and viscosities before stirring (V) and after stirring (Vs) of gels at various stages of formation by acidification of reconstituted NDM (10%, wt/vol) with 2% glucono-δ-lactone at 25°C.[1]

	Incubation time												
	0 h	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	3.5 h	4.5 h	6.0 h	7.5 h	9.0 h	10.5 h	24.0 h
V, mPa.s	10	10	10	10	10	40	45	50	60	70	75	80	80	90
Vs, mPa.s	10	10	10	10	10	30	30	40	50	50	60	65	70	70
pH	6.4	5.5	5.2	5.1	5.0	4.9	4.7	4.6	4.5	4.5	4.3	4.3	4.3	4.0

[1]Means of two measurements; the maximum deviation between duplicate results was 10%.

slightly and reached values of only 100 mPa.s. The gels were lumpy, and syneresis was apparent. The chemically acidified gel was fragile and reached a viscosity of only 90 mPa.s at pH 4.0 (Table 1), which is close to values obtained with the nonproducing strains.

In another set of experiments, viscosities were measured before and after stirring (Table 2). In that series, the overall viscosities of the St/Lb 1187 culture measured before stirring were lower than those shown in Figure 2, which illustrates the difficulties in obtaining reproducible results in viscosity and EPS yield because of the instability of the EPS-producing trait of our strain. Similar observations on the instability of the EPS-producing ability and concurrent lower capacities for promoting texture have been made with other lactic acid bacteria (7, 17, 21). Lower viscosities were associated with less EPS production. The viscosities measured after stirring were lower than those measured before stirring. However, even after stirring, the viscosities obtained with the EPS-producing strains remained higher (between 170 and 230 mPa.s) than those measured in milks fermented with nonproducing strains or in glucono-δ-lactone acidified gels (Table 1). The stirring technique cannot be compared with industrial conditions, and, therefore, conclusions cannot be drawn for yogurt manufacture, but the comparison of viscosities measured before and after stirring is nonetheless instructive.

EPS Production

The St/Lb W culture produced the highest amount of EPS (240 mg/L), the St/Lb 1187 culture produced the lowest (110 mg/L), and the St/Lb P culture an intermediate amount of EPS (200 mg/L) (Figure 3). Production of EPS from the St/Lb W and St/Lb P cultures began earlier and was higher after 3 h of fermentation; almost half of the final EPS (120 mg/L) was obtained after 4.5 h of incubation. The EPS from the St/Lb 1187 culture was almost at the maximum (90 mg/L) at the beginning of the stationary

growth phase, but EPS from the St/Lb P and St/Lb W cultures continued during the early stationary growth phase. The EPS yield from the St/Lb P culture decreased considerably from 200 to 150 mg/L between 10.5 and 24 h of fermentation. No direct relationship appeared between viscosities and EPS amounts (i.e., the highest viscosity values did not correspond to the highest EPS yield). The physicochemical characteristics of the EPS from mixed-strain cultures possibly were different from those of EPS from single-strain cultures.

Sugar Composition

The EPS that were produced by the St/Lb 1187, St/Lb W, and St/Lb P cultures were neutral heteropolysaccharides. They contained mainly galactose and smaller amounts of glucose and rhamnose. Galac-
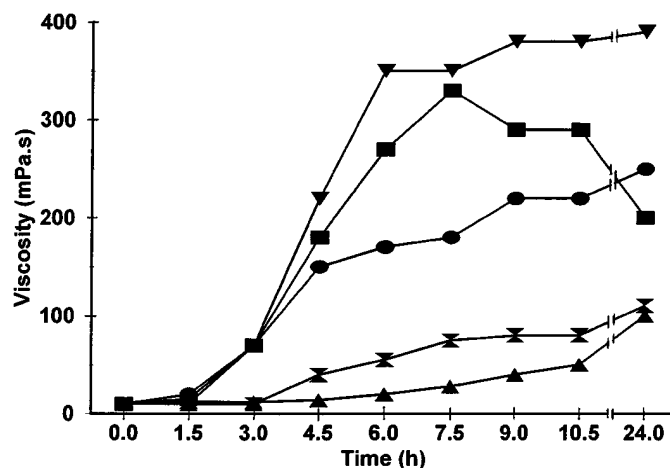

Figure 2. Viscosities of reconstituted NDM (10% wt/vol) during three mixed-strain culture fermentations at 42°C by the non-exopolysaccharide (EPS)-producing *Streptococcus thermophilus* CNRZ 389 with the parental EPS-producing strain *Lactobacillus delbrueckii* ssp. *bulgaricus* CNRZ 1187 (St/Lb 1187) (▼), with the white (W) (●) and with the pink (P) (■) colonial variants, and two single-strain fermentations of *S. thermophilus* CNRZ 389 (▲) and non-EPS-producing *L. delbrueckii* ssp. *bulgaricus* CNRZ 398 (double triangle). The values are the means of two measurements, and the maximum deviation between duplicate results is 10%.

TABLE 2. Viscosities[1] before stirring (V) and after stirring (Vs) of gels formed by fermentation of reconstituted NDM (10%, wt/vol) at 42°C by three mixed-strain cultures: non-EPS[2]-producing *Streptococcus thermophilus* CNRZ 389 with the parental EPS-producing strain *Lactobacillus delbrueckii* ssp. *bulgaricus* (St/Lb 1187), with the white (W) and with the pink (P) colonial variants, and two single-strain cultures: non-EPS-producing *S. thermophilus* CNRZ 389 and *L. delbrueckii* ssp. *bulgaricus* CNRZ 398.

Time	St/Lb 1187		St/Lb W		St/Lb P		CNRZ 389		CNRZ 398	
	V	Vs	V	Vs	V	Vs	V	Vs	V	Vs
(h)					(mPa.s)					
0.0	10	10	10	10	10	10	10	10	10	10
1.5	10	10	20	20	15	10	10	10	10	10
3.0	50	30	70	40	70	60	10	10	10	10
4.5	210	200	150	110	180	170	10	10	40	30
6.0	280	200	170	160	270	250	20	10	55	30
7.5	300	210	180	180	330	200	40	20	75	40
9.0	310	230	220	200	290	200	40	20	80	50
10.5	310	230	220	180	290	220	50	30	80	50
24.0	310	230	250	230	200	170	100	50	110	70

[1]Means of two measurements; the maximum deviation between duplicate results was 10%.

[2]Exopolysaccharide.

tose predominated in EPS during the entire fermentation cycle, comprising approximately 50% of the total sugars (Table 3). Proportions of glucose varied from 14% (1.5 h) to almost 22% for EPS from St/Lb 1187. The variations were similar for EPS from St/Lb W and different for EPS from St/Lb P; glucose accounted for 27% of total sugars after 1.5 h and 24% after 24 h of incubation. Small amounts of arabinose, decreasing from about 9 to 2% of the total, were present also. The most striking trends were the rhamnose and mannose concentrations in the EPS from St/Lb 1187. Rhamnose was present in trace amounts at the beginning of the fermentation but represented 15.5% after 24 h; mannose decreased from an initial 23% to around 4% at 24 h. Rhamnose levels increased even faster in EPS from St/Lb P and St/Lb W, and the proportions of mannose were lower in the early growth phase compared with EPS from St/Lb 1187. The proportions of the monosaccharides changed only during the logarithmic growth phase (6 h) and remained stable thereafter.

DISCUSSION

Generally, yogurt is made with *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus,* and the association between the two organisms, termed protocooperation, has been studied in terms of metabolic interactions (2, 26, 29, 36). Less is known about the influence of protocooperation in mixed-strain starter cultures on EPS production, EPS composition, and improvement of texture. Our previous work (5) showed that the heterogeneous cell constitution of *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 is correlated with EPS produc-

tion and the ability to improve texture in single-strain cultures. The potential influence of this variability in mixed-strain cultures with *S. thermophilus* has not been investigated yet.

The general parameters—such as growth, acid development, and sugar utilization—were determined to control the fermentation cycle, but these variables
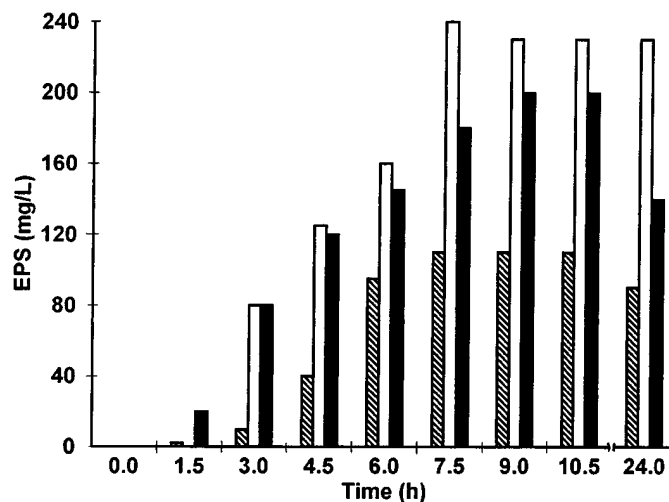

Figure 3. Exopolysaccharides (EPS) produced during three mixed-strain culture fermentations on the non-EPS-producing *Streptococcus thermophilus* CNRZ 389 with the parental EPS-producing strain *Lactobacillus delbrueckii* ssp. *bulgaricus* CNRZ 1187 (St/Lb 1187) (patterned bar), with the white (W) (□) and with the pink (P) (■) colonial variants in reconstituted NDM (10%, wt/vol) at 42°C. The values are the means of two measurements, and the maximum deviation between duplicate results is 10%.

were less important for EPS production and viscosity than was the phenotype of *L. delbrueckii* ssp. *bulgaricus* used in mixed-strain cultures.

The mixed-strain cultures of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* we studied produced acid at a faster rate and to a greater extent than either of the two bacteria separately, which is in agreement with results of Rajagopal and Sandine (26). All mixed-strain cultures reached a maximum cell count after 6 h of fermentation, which is close to results obtained with single-strain cultures of *L. delbrueckii* ssp. *bulgaricus* (5). Compared with the same single-strain cultures (5), lactose utilization was faster, and the final lactose concentration was lower, in the mixed-strain cultures with *L. delbrueckii* ssp. *bulgaricus*. Concurrently, galactose concentration was higher in the latter, which is comparable with results from Oner and Erickson (25).

In the present work, all three mixed-strain cultures produced higher viscosities than the single-strain cultures. But, in our previous study on single-strain cultures with *L. delbrueckii* ssp. *bulgaricus* and the two colonial variants, the relationship between EPS yield and viscosity was fairly good, and the highest EPS production corresponded to the highest viscosity (5). The situation is very different in the present study with mixed-strain cultures, because here the EPS yield was not correlated with viscosity. This lack of correlation is particularly evident regarding results obtained with the mixed-strain cultures containing the parental strain *L. delbrueckii* ssp. *bulgaricus* and the W variant. The first produced 110 mg EPS/L for a viscosity of 390 mPa.s, and the second produced 240 mg EPS/L for a viscosity of 220 mPa.s. These results are not easy to interpret, but they show that the amount of EPS produced is not the only factor to be considered. It is possible, that EPS produced in mixed-strain cultures has different physicochemical characteristics, such as molecular mass and thickening properties, than EPS produced in single-strain cultures. Further research is needed for confirmation.

It is well known that pH has a considerable influence on the structure of the coagulum formed by milk proteins and that amounts of EPS are not the sole factor affecting the viscosity and texture of fermented milk. However, when the viscosity readings at 6 h in Figure 2 (170, 270, and 350 mPa.s for St/Lb W, St/Lb P, and St/Lb 1187 respectively) are compared with the corresponding pH values (4.1, 4.0 and 4.1), it is obvious that the observed viscosities were affected primarily by secreted EPS. At pH 4.0, the chemically acidified gel had a viscosity of 90 mPa.s, and cultures that did not produce EPS had a viscosity of approximately 100 mPa.s.

Measurements before and after stirring (Table 2) showed that viscosities of the milks fermented with EPS-producing strains remained much higher (170 to 230 mPa.s), than those obtained with nonproducing strains (50 to 70 mPa.s) and with chemically acidified gels (70 mPa.s; Table 1). This result shows the

TABLE 3. Relative monosaccharide composition of exopolysaccharides (EPS)[1] produced during the three mixed-strain culture fermentations of the non-EPS-producing *Streptococcus thermophilus* CNRZ 389 with the parental EPS-producing strain *Lactobacillus delbrueckii* ssp. *bulgaricus* CNRZ 1187 (St/Lb 1187), with the white (W) and with the pink (P) colonial variants, at different incubation times in reconstituted NDM (10%, wt/vol) at 42°C.

Mixed-strain culture	Sugar	Incubation time							
		1.5 h	3.0 h	4.5 h	6.0 h	7.5 h	9.0 h	10.5 h	24.0 h
		(%)							
St/Lb 1187	Rhamnose	0.8	7.3	15.6	14.6	13.4	12.2	11.6	15.5
	Arabinose	9.1	5.7	3.0	2.1	1.7	2.0	2.3	2.4
	Mannose	23.1	15.3	6.2	5.7	4.6	4.1	4.6	4.4
	Galactose	49.4	48.2	49.6	50.1	52.2	53.4	53.7	49.7
	Glucose	14.0	17.4	18.0	21.0	22.2	21.7	20.4	21.7
St/Lb W	Rhamnose	ND[2]	25.1	25.8	24.4	22.2	21.6	20.8	20.5
	Arabinose	ND	2.6	2.0	2.0	1.6	2.0	1.6	2.0
	Mannose	ND	3.4	4.0	3.5	3.7	2.9	3.6	2.8
	Galactose	ND	48.7	46.3	47.5	49.6	50.3	48.1	47.5
	Glucose	ND	13.0	14.0	15.8	16.0	16.7	17.2	17.9
St/Lb P	Rhamnose	2.0	23.8	17.9	19.3	16.4	15.2	16.3	17.1
	Arabinose	8.3	2.9	2.1	1.8	1.8	1.7	1.5	1.5
	Mannose	9.3	5.0	4.6	2.5	3.4	3.6	3.0	3.3
	Galactose	52.8	45.1	49.5	47.3	50.3	49.6	48.4	48.0
	Glucose	27.3	12.9	18.6	21.7	20.2	23.5	23.1	23.6

[1]Means of two measurements; the maximum deviation between duplicate results was 10%.

[2]Not determined.

advantage of using EPS-producing strains in the manufacture of stirred yogurt. Our observations are in agreement with studies on the rheology and microstructure of ropy yogurt showing that ropy cultures exhibit less pseudoplastic behavior than do nonropy cultures; that is, the viscosities of the cultures decrease less at higher shear rates (27, 28, 30, 31). Interestingly, the milk fermented with St/Lb W culture, which had the highest EPS yield and the lowest viscosity before stirring, was more resistant to stirring than were the others. This result may also indicate that the EPS from ST/Lb W had different physicochemical properties.

The EPS production by St/Lb P began earlier (1.5 h) than did that of St/Lb W and St/Lb 1187. Production of EPS by St/Lb 1187 was almost achieved at the end of the exponential growth phase (6 h), and production of EPS by St/Lb W was achieved during the early stationary phase (7.5 h). Only EPS production from St/Lb P continued during the stationary phase and then decreased sharply between 10.5 and 24 h with concurrent viscosity decrease. This result would indicate the presence of enzymes that are capable of degrading EPS, as has been suggested for other lactic acid bacteria, such as *S. thermophilus* (7, 15). Compared with single-strain cultures (5), the behavior of the P and W phenotype was reversed in mixed-strain cultures with *S. thermophilus*. In terms of EPS yield, the W variant was the highest EPS producer, and the P variant was the lowest. The faster EPS production in mixed-strain cultures than in single-strain cultures may be of interest for the manufacture of yogurt.

The EPS produced by the mixed-strain St/Lb 1187, St/Lb W, and St/Lb P cultures were neutral heteropolysaccharides composed mainly of galactose and smaller amounts of glucose and rhamnose. The proportions of the component sugars varied only during the exponential growth phase (6 h) and remained stable thereafter. This result is different from that observed for EPS from the single-strain *L. delbrueckii* ssp. *bulgaricus* CNRZ 1187 (5) for which the composition varied during the longer fermentation time (10 h). Also, rhamnose, which is absent in EPS from single-strain *L. delbrueckii* ssp. *bulgaricus* cultures (5), is present in all EPS isolated from mixed-strain cultures containing *S. thermophilus*. The monosaccharide composition of EPS can depend on the carbon source available in the medium (9), and the regulation of the biosynthetic pathway of EPS in *L. delbrueckii* ssp. *bulgaricus* may be dependent on the carbon source (19). It has not been shown yet that EPS with different sugar composition and, hence,

different structures can be produced in the same medium and with the same carbon source (i.e., lactose from milk) in mixed-strain cultures with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*. It has been shown that EPS isolated from other pure strain cultures of *L. delbrueckii* ssp *bulgaricus* contain glucose, galactose, and rhamnose (6, 20), but it is not yet understood why variability in EPS composition occurs at strain level. However, this variability would mean that some of the enzymes leading to the synthesis of sugar nucleotides involved in EPS synthesis in milk are absent in single-strain cultures and present in mixed-strain cultures because of the presence of *S. thermophilus*. More work needs to be done to understand fully how EPS synthesis is controlled genetically and biochemically.

We have shown that EPS in mixed-strain cultures was produced faster than in single-strain cultures and that the composition of the EPS is very different. It is not possible at this time to determine definitely whether EPS from the different phenotypes of *L. delbrueckii* ssp. *bulgaricus* associated with *S. thermophilus* have different physicochemical properties even though the hypothesis of varying molecular masses seems reasonable. In particular, more work is necessary on the properties of EPS (i.e., molecular mass and intrinsic viscosity) to understand better the role of EPS in yogurt manufacture.

ACKNOWLEDGMENTS

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