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Carbon Source Requirements for Exopolysaccharide Production by *Lactobacillus casei* CG11 and Partial Structure Analysis of the Polymer

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Exopolysaccharide production by *Lactobacillus casei* CG11 was studied in basal minimum medium containing various carbon sources (galactose, glucose, lactose, sucrose, maltose, melibiose) at concentrations of 2, 5, 10, and 20 g/liter. *L. casei* CG11 produced exopolysaccharides in basal minimum medium containing each of the sugars tested; lactose and galactose were the poorest carbon sources, and glucose was by far the most efficient carbon source. Sugar concentrations had a marked effect on polymer yield. Plasmid-cured Muc$^-$ derivatives grew better in the presence of glucose and attained slightly higher populations than the wild-type strain. The values obtained with lactose were considerably lower for both growth and exopolysaccharide yield. The level of specific polymer production per cell obtained with glucose was distinctively lower for Muc$^-$ derivatives than for the Muc$^+$ strain. The polymer produced by *L. casei* CG11 in the presence of glucose was different from that formed in the presence of lactose. The polysaccharide produced by *L. casei* CG11 in basal minimum medium containing 20 g of glucose per liter had an intrinsic viscosity of 1.13 dl/g. It was rich in glucose (76%), which was present mostly as 2- or 3-linked residues along with some 2,3 doubly substituted glucose units, and in rhamnose (21%), which was present as 2-linked or terminal rhamnose; traces of mannose and galactose were also present.

Many dairy lactic acid bacteria used for manufacturing fermented milk products can produce exocellular polysaccharides (EPS), which are excreted into the milk during fermentation, prevent syneresis, and ensure proper texture and body of the end product. Furthermore, it has been claimed that EPS isolated from lactic acid bacterial cultures have antitumor activity (25). Dairy starters containing slime-forming *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* for making yoghurt (6–10, 14, 27, 30–32) and mesophilic starters (10, 13, 20, 21, 24, 26, 27, 31) containing polymer-producing *Lactococcus lactis* subsp. *cremoris* for making Scandinavian ropy sour milk products, such as "viili" and "longfil", are available in Europe and the United States. These lactic acid bacteria produce EPS containing glucose, galactose, and often rhamnose.

The quantities of EPS produced in milk by different species and strains vary considerably; the amounts of EPS reportedly range from 50 to 350 mg/l for *S. thermophilus* (8, 12), from 60 to 150 mg/l for *Lactobacillus bulgaricus* (7, 14), and from 80 to 600 mg/l for *Lactococcus lactis* subsp. *cremoris* (10). The EPS have apparent molecular weights that range from 5×10^5 (*Lactobacillus bulgaricus*) (7) to 1.7×10^6 (*Lactococcus lactis* subsp. *cremoris*) (24) and to 1×10^6 (*S. thermophilus*) (12). Some of the EPS exhibit remarkable thickening properties and have high intrinsic viscosities (6, 12). The structures of the repetitive sequences of the EPS produced by some dairy lactic acid bacteria, including *S. thermophilus* (12), *Lactobacillus bulgaricus rr* (16), and *Lactococcus lactis* subsp. *cremoris* H414 and SBT0495 (15, 23), have been elucidated previously.

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Contradictory results have been reported regarding the influence of stimulating factors on EPS production. Enhanced EPS production and growth were obtained when hydrolyzed casein was added to skim milk cultures of *Lactobacillus bulgaricus* (14). According to Schellhaass (27), neither growth nor EPS production was specifically linked to the presence of casein or whey proteins in the growth medium of thermophilic and mesophilic lactic acid bacteria. However, Cerning et al. found that casein stimulates EPS production but not growth of *Lactobacillus bulgaricus* (7). It has also been reported that *Lactobacillus bulgaricus* is able to produce the same amount of EPS in milk and milk ultrafiltrate, but that *S. thermophilus* is not (9). On the other hand, supplementation of milk ultrafiltrate with glucose or sucrose stimulates EPS production by *Lactobacillus casei* and modifies the composition of the EPS (10).

It has been shown recently (19) that *Lactobacillus casei* CG11 is able to produce EPS in a chemically defined medium supplemented with fermentable sugar. In addition, plasmid-cured derivatives of this strain having the nonmucoid phenotype Muc$^-$ have been isolated. Compared with milk and milk ultrafiltrate, a synthetic medium allows faster and easier isolation of EPS and facilitates the study of the influence of individual components of the medium (e.g., the influence of sugars on EPS production).

In this work an EPS-producing strain of *Lactobacillus casei* strain CG11, was studied to assess the carbon source requirements for EPS production. Plasmid-cured derivatives were compared with the parental strain with respect to residual polymer production and composition of the EPS. The structure of the EPS produced by the parental strain in a chemically

defined medium containing glucose as the sole carbon source was also partially analyzed.

MATERIALS AND METHODS

Microorganisms. *Lactobacillus casei* CG11 (Muc^+) and plasmid-cured Muc^- derivatives obtained after treatment with novobiocin (strain CG11-NB) and a sublethal temperature (strain CG11-ST) were made resistant to streptomycin. Streptomycin-resistant derivatives were obtained spontaneously by plating 0.2-ml aliquots of overnight cultures (cultures grown for 16 h) onto MRS plates containing 0.5 mg of streptomycin. After 48 h of incubation at 30°C, streptomycin-resistant colonies of strain CG11 and the Muc^- derivatives CG11-NB and CG11-ST were isolated and subjected to analysis. The streptomycin-resistant mutant of strain CG11 produced EPS, whereas the streptomycin-resistant mutants of CG11-NB and CG11-ST did not; all three mutants exhibited resistance to up to 1 mg of streptomycin per ml.

Media and growth conditions. The organisms were grown in basal minimum medium (BMM) containing amino acids, minerals, and vitamins (22) or in milk ultrafiltrate (Bongrain, Lyon, France) supplemented with 1% saccharide-free Casamino Acids (Difco Laboratories, Detroit, Mich.). To study the influence of various carbon sources on EPS production by strain CG11, galactose, glucose, lactose, sucrose, maltose, and melibiose were added to BMM at concentrations of 2, 5, 10, and 20 g/liter. The media always contained streptomycin (1 mg/ml), which allowed permanent monitoring of the cultures during the experiment. The media were sterilized by heating them at 110°C for 15 min without the sugars, which were filter sterilized. Fermentation cultures inoculated with precultures (10%, vol/vol) that had been grown in the same media as the fermentation cultures were incubated in duplicate at 25°C for 48 h. After incubation the cultures were cooled in ice water, and the numbers of cells were determined microscopically by counting the individual cells in 20 to 30 fields after methylene blue staining by Breed's method; the resulting values were expressed as direct microscopic counts (DMC) (1).

EPS isolation and purification. After incubation, the cultures were heated at 100°C for 15 min to inactivate the enzymes capable of degrading the polymer, and the cells were removed by centrifugation at 16,000 × *g* for 30 min at 4°C. EPS were precipitated by adding 3 volumes of chilled 100% ethanol. The precipitate was collected by centrifugation at 12,000 × *g* for 20 min at 4°C and dissolved in distilled water. After dialysis against water for 24 h at 4°C (molecular weight cutoff for the dialysis membranes, 2,000 to 3,000), the solution was freeze dried. When this isolation procedure was used, the level of relative error between two trials was between 5 to 10%. At this state, the crude EPS preparations contained between 20 and 30% total sugars.

For the partial structure analysis, the crude EPS was further purified by dissolving the crude preparation in 10% trichloroacetic acid, and the precipitate was washed twice with 10% trichloroacetic acid in order to recover the total polymer. After exhaustive dialysis for 5 days with at least three water changes per day, the solution was freeze dried; the resulting preparation contained 65% sugars.

Neutral sugars. The total sugar concentration was determined by the phenol-sulfuric method, using glucose as a standard (11). The component sugars were qualitatively identified after acid hydrolysis (2 N trifluoracetic acid, 16 h, 100°C) by thin-layer chromatography and were visualized with diphenyl-aniline. For quantitative analysis of the sugars, the EPS was hydrolyzed with 2 M trifluoracetic acid at 120°C for 2.5 h. When a precipitate appeared upon alkalinization, it was eliminated by centrifugation before derivatization of the component sugars to alditol acetates as described by Blakeney et al. (3). The alditol acetate contents were determined (using inositol as an internal standard) by gas-liquid chromatography on a fused-silica capillary column (30 m by 0.32 mm) bonded with OV-225; the column temperature was 210°C, the injector temperature was 210°C, the detector temperature was 240°C, the split rate was 60 to 80 ml/min, and the carrier gas was hydrogen at a pressure of 0.7×10^5 Pa.

Structure analysis. A methylation analysis was performed by the method of Hakomori (17), using lithium methylsulfinyl anion and a contact time of 1 h. Methylated polysaccharides were extracted with $CHCl_3$-CH_3OH (2:1, vol/vol), washed three times with distilled water, air dried at 40°C, and then hydrolyzed with 2 M trifluoroacetic acid for 90 min at 120°C. The resulting compounds were converted to their alditol acetates by using perchloric acid as a catalyst (18) and were analyzed by gas-liquid chromatography on (i) a fused-silica capillary column (30 by 0.32 mm) bonded with OV-225 (temperature kept at 175°C for 15 min and then increased at a rate of 5°C/min to 220°C; injector temperature, 210°C; detector temperature, 240°C; split rate, 60 to 80 ml/min; carrier gas, hydrogen at a pressure of 0.7×10^5 Pa) and (ii) a fused-silica capillary column (30 m by 0.32 mm) bonded with OV-1 (temperature kept at 150°C for 10 min and then increased at a rate of 2°C/min to 190°C; injector temperature, 210°C; detector temperature, 240°C; split rate, 60 to 80 ml/min; carrier gas, hydrogen at a pressure of 0.7×10^5 Pa). Peaks were identified on the basis of retention times by using inositol as an internal standard and on the basis of electron impact fragmentation patterns by using the OV-225 column coupled to a Delsi-Nermag R10-10C mass spectrometer with a source temperature of 250°C. The compounds were quantified by using the effective carbon responses determined by Sweet et al. (29).

Intrinsic viscosity. Intrinsic viscosity was calculated by measuring the flow rates of EPS solutions in 0.155 M NaCl at 25 ± 0.1°C with an automatic Ubbellohde viscosimeter (Amtec, Nice, France) and by using the double extrapolation to zero concentration based on the equations of Huggins and Kramer (2).

RESULTS

Effects of various carbon sources on pH, growth, and EPS yield. To determine the effects of various carbon sources and concentrations on the yield of EPS produced, *Lactobacillus casei* was grown at 25°C for 48 h in BMM supplemented with galactose, glucose, sucrose, maltose, or melibiose at concentrations of 2, 5, 10, and 20 g/liter (Table 1). The pH values decreased from 6.2 to 5.8 after 48 h of incubation in BMM supplemented with 20 g of galactose per liter and from 6.5 to 6.1 when 20 g of lactose per liter was added to the medium. With melibiose the pH remained almost constant for 48 h of incubation independent of the sugar concentration, and with sucrose and maltose the pH of the medium decreased (from 6.2 to 5.0 with 20 g of sucrose per liter and from 6.3 to 5.2 with 20 g of maltose per liter). The decrease in pH from 6.0 to 4.7 observed with 20 g of glucose per liter in the medium was the most important decrease.

Mesophilic lactic acid bacteria normally produce populations of 4×10^9 to 5×10^9 DMC when they are grown at 30°C; the lower incubation temperatures used in this study to enhance EPS production resulted in somewhat less growth. The lowest numbers of cells were obtained with galactose (6×10^7 to 1.0×10^8 DMC) and lactose (5.9×10^6 to 3.3×10^7 DMC),

TABLE 1. Effects of various carbon sources on pH, growth, and EPS yield obtained with *Lactobacillus casei* CG11 after 48 h of incubation at 25°C in BMM containing sugars at concentrations of 2, 5, 10, and 20 g/liter[a]

Sugar	Concn (g/liter)	pH		Cell no. (DMC/ml)[b]		EPS yield (mg/liter)[c]
		Initial	Final	Initial	Final	
Galactose	2	6.2	6.0	1.5×10^7	9.8×10^7	10
	5	6.2	6.0	1.6×10^7	7.5×10^7	10
	10	6.2	6.0	1.6×10^7	6.0×10^7	15
	20	6.2	5.8	1.5×10^7	1.0×10^8	20
Glucose	2	6.0	5.3	8.7×10^7	4.4×10^8	70
	5	6.0	4.8	8.1×10^7	1.4×10^9	100
	10	6.0	4.7	9.5×10^7	9.7×10^8	120
	20	6.0	4.7	1.0×10^8	7.9×10^8	160
Lactose	5	6.5	6.2	4.1×10^6	5.9×10^6	10
	10	6.5	6.2	3.0×10^6	7.9×10^6	15
	20	6.5	6.1	4.7×10^6	3.3×10^7	45
Sucrose	2	6.2	5.2	4.5×10^7	7.2×10^8	35
	5	6.2	5.0	5.0×10^7	7.6×10^8	40
	10	6.2	5.0	6.5×10^7	1.0×10^9	45
	20	6.2	5.0	5.1×10^7	5.6×10^8	50
Maltose	2	6.3	5.2	1.1×10^7	8.5×10^8	15
	5	6.3	5.2	1.2×10^7	9.5×10^8	20
	10	6.3	5.0	1.1×10^7	7.0×10^8	25
	20	6.3	5.2	9.5×10^6	7.5×10^8	60
Melibiose	2	6.2	6.1	7.0×10^5	3.6×10^7	15
	5	6.2	6.1	3.1×10^6	4.7×10^7	20
	10	6.2	6.1	4.2×10^6	4.2×10^7	35
	20	6.2	6.0	3.2×10^6	5.5×10^7	100

[a] The values are the means of two measurements.
[b] The maximum deviation between duplicate results was 10%.
[c] The maximum deviation between duplicate results was 5%.

while slightly higher cell numbers were observed with glucose (4.4×10^8 to 1.4×10^9 DMC), sucrose (5.6×10^8 to 1.0×10^9 DMC), and maltose (7.0×10^8 to 9.5×10^8 DMC). However, increasing the sugar concentrations from 2 to 20 g/liter did not affect growth.

Even at concentrations of 20 g/liter, galactose and lactose gave the lowest EPS yields (20 and 45 mg of EPS per liter, respectively). With melibiose, the EPS yield increased dramatically from 15 to 100 mg of EPS per liter when the sugar concentration was increased from 2 to 20 g/liter. With glucose, sucrose, and maltose the EPS yield increased when the sugar concentration in the medium increased. The EPS yield obtained with 2 g of sucrose per liter was 35 mg/liter, and the EPS yield obtained with 20 g of sucrose per liter was 50 mg/liter. When the maltose concentration was increased from 2 to 20 g/liter, the EPS yield increased from 15 to 60 mg/liter. With 2 g of glucose per liter, the EPS yield was 70 mg/liter, which was greater than the yield obtained with any other carbon source at the same concentration. When the medium contained 20 g of glucose per liter, the amount of EPS produced was 160 mg/liter; thus, glucose was by far the most efficient carbon source in terms of EPS production.

Comparative analysis of EPS production. To check the ability of *Lactobacillus casei* CG11 and its plasmid-cured Muc$^-$ derivatives (strains CG11-NB and CG11-ST) to produce EPS under various conditions, these organisms were incubated in milk ultrafiltrate supplemented with 1% Casamino Acids and in BMM containing either 20 g of glucose per liter or 20 g of lactose per liter for 48 h at 25°C. Since the two Muc$^-$ derivatives were produced by different plasmid-curing treatments (19), both were used in this analysis to check whether the curing treatment itself had an influence on EPS production (Table 2).

In BMM supplemented with 20 g of glucose per liter plasmid-cured derivatives CG11-NB and CG11-ST grew faster and produced slightly larger populations (1.8×10^9 and 5.7×10^9 DMC, respectively) than parental strain CG11 (9.1×10^8 DMC). The EPS yield was 130 mg/liter for CG11, whereas it was only 15 mg/liter for CG11-NB and 30 mg/liter for CG11-ST. In addition, the level of specific EPS production was 16 times higher in strain CG11 (1.4×10^{-10} mg of EPS per DMC) than in the Muc$^-$ derivatives (0.09×10^{-10} mg of EPS per DMC).

When the organisms were grown in BMM containing 20 g of lactose per liter or in milk ultrafiltrate containing 30 g of lactose per liter, the values obtained for both growth and EPS yield were considerably lower. There was less acid production in milk ultrafiltrate and in BMM; only strain CG11 exhibited a slight decrease in pH (pH 6.3 to 5.9). For the Muc$^-$ derivatives, the pH remained constant during incubation. The cell numbers were extremely low for Muc$^-$ derivatives grown in BMM supplemented with 20 g of lactose per liter (6.6×10^6 to 7.3×10^6 DMC); the cell numbers were a little higher for organisms grown in milk ultrafiltrate supplemented with 1% Casamino Acids (5.3×10^7 to 9.7×10^7 DMC). Strain CG11

TABLE 2. pH, growth, and EPS yield obtained with *Lactobacillus casei* CG11 and plasmid-cured Muc$^-$ derivatives CG11-NB and CG11-ST in BMM containing glucose or lactose as a carbon source or in milk ultrafiltrate after 48 h of incubation at 25°C[a]

Organism	Medium	Carbon source	pH		Cell no. (DMC/ml)[b]		EPS yield (mg/liter)[c]
			Initial	Final	Initial	Final	
CG11	BMM	Glucose	6.3	4.5	3.9×10^6	9.1×10^8	130
CG11-NB			6.2	4.4	8.2×10^6	1.8×10^9	15
CG11-ST			6.2	4.3	7.7×10^6	5.7×10^9	30
CG11	BMM	Lactose	6.3	5.9	3.3×10^5	2.0×10^6	35
CG11-NB			6.2	6.2	2.6×10^6	7.3×10^6	30
CG11-ST			6.3	6.2	1.6×10^6	6.6×10^6	20
CG11	Milk ultrafiltrate		6.2	5.8	3.3×10^6	3.8×10^7	40
CG11-NB			6.1	5.8	3.3×10^6	5.3×10^7	30
CG11-ST			6.1	5.7	4.0×10^6	9.7×10^7	35

[a] The values are the means of two measurements.
[b] The maximum deviation between duplicate results was 10%.
[c] The maximum deviation between duplicate results was 5%.

TABLE 3. Relative monosaccharide compositions of the EPS produced by parental *Lactobacillus casei* CG11 and Muc$^-$ derivatives CG11-NB and CG11-St in BMM containing glucose (20 g/liter) or lactose (20 g/liter) as a carbon source after 48 h of incubation at 25°C[a]

Organism	Carbon source	Composition of EPS (%)[b]						
		Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
CG11	Lactose	16.1	0	0	1.0	6.8	13.2	63.0
	Glucose	20.5	0	0	0	1.7	2.1	75.7
CG11-NB	Lactose	1.9	1.9	1.0	0	10.4	47.1	37.0
	Glucose	3.1	0	0	0	6.2	4.1	86.6
CG11-ST	Lactose	2.1	1.4	0	0	7.5	49.7	39.2
	Glucose	2.2	0	0	0	5.5	4.2	88.0

[a] The values are the means of two measurements.
[b] The maximum deviation between duplicate results was 1%.

grew better in milk ultrafiltrate (3.8×10^7 DMC) than in BMM supplemented with lactose (2.0×10^6 DMC), and the strain CG11 EPS yields obtained with ultrafiltrate and BMM were low (35 to 40 mg/liter) and were similar to values reported previously (10). The EPS yields obtained with the Muc$^-$ were very similar. However, the level of specific EPS production was lower in ultrafiltrate with CG11-NB or CG11-ST (5.7×10^{-10} to 3.6×10^{-10} mg of EPS per DMC) than in ultrafiltrate with strain CG11 (11.1×10^{-10} mg of EPS per DMC).

Sugar composition. The sugar compositions of EPS produced by the organisms in BMM containing glucose or lactose were analyzed after acid hydrolysis. The EPS formed by strain CG11 and the plasmid-cured Muc$^-$ derivatives in BMM containing glucose as the sole carbon source contained mainly glucose. The concentration of glucose in the EPS formed by the Muc$^-$ derivatives was higher (86.6 to 88%) than the concentration of glucose in the EPS formed by strain CG11 (75.6%). Small amounts of galactose were present in the EPS produced by all three strains (Table 3).

In contrast, the monosaccharide composition of the EPS produced in BMM containing only lactose as a carbon source was different with respect to glucose. Glucose accounted for 63% of the EPS formed by strain CG11, whereas glucose accounted for much less of the EPS formed by Muc$^-$ derivatives CG11-NB (37%) and CG11-ST (39.2%). Furthermore, the amount of galactose was greater in the EPS produced in BMM containing lactose than in the EPS produced in BMM containing glucose. The level of galactose was higher in the EPS produced by Muc$^-$ derivatives CG11-NB (47.1%) and CG11-ST (49.7%) than in the EPS produced by strain CG11 (13.2%). Small amounts of mannose were also found in the EPS produced by all three strains (Table 3).

The most significant difference in the monosaccharide compositions of the EPS produced by parental strain CG11 and the Muc$^-$ derivatives occurred in the rhamnose content; the rhamnose content was approximately 10 times higher in the EPS formed by strain CG11 than in the EPS produced by Muc$^-$ derivatives CG11-NB and CG11-ST, regardless of the carbon source (glucose or lactose) in which the EPS was synthesized. In contrast, small amounts of fucose were detected in the EPS produced by the Muc$^-$ derivatives in the presence of lactose; no fucose was detected in the EPS produced by these strains in the presence of glucose. Fucose was not present in the EPS produced by strain CG11 in the presence of either glucose or lactose (Table 3).

A methylation analysis of the EPS obtained from CG11 grown in BMM supplemented with 20 g of glucose per liter showed that there were three main products, 3,4-dimethyl-1,2,5-triacetyl rhamnitol, 2,4,6-trimethyl-1,3,5-triacetyl glucitol, and 3,4,6-trimethyl-1,2,5-triacetyl glucitol, each of which accounted for about one-fourth of the sugar ethers (26, 24, and 28%, respectively). The minor derivatives which could be detected were 4,6-dimethyl-1,2,3,5-tetraacetyl glucitol (13%), 2,3,4-trimethyl-1,5-diacetyl rhamnitol (2%), 3,4-dimethyl-1,2,5,6-tetraacetyl glucitol (2%), and nonmethylated rhamnose (3%) and glucose (2%). Thus, the EPS was composed mostly of 3- and 2-linked glucose residues and 2-linked rhamnose residues, as well as a few doubly substituted glucose residues. Only rhamnose was found as a nonreducing endgroup, and the proportion of nonreducing end groups was low. This may have been due to the presence of nonsugar substituents on the EPS or undermethylation or both. The EPS, which had an intrinsic viscosity of 1.13 dl/g, exhibited thickening properties, but they were not extreme.

DISCUSSION

The total yield of EPS produced by lactic acid bacteria depends on the composition of the medium and the conditions in which the organisms grow (i.e., temperature and incubation time) (7–10). Similar observations have been made for gram-negative bacteria, including *Klebsiella* sp., *Acinetobacter calcoaceticus* (5), and *Aeromonas salmonicida* (4). Our results show that both the yield and the composition of the EPS produced by *Lactobacillus casei* CG11 depend on the carbon source present in the medium. Glucose was the most efficient carbon source for EPS production, whereas lactose was not an efficient carbon source. The sugar concentration had a marked effect on EPS yield. For instance, raising the glucose concentration resulted in increased EPS production; the maximum EPS production occurred with 20 g of glucose per liter in the medium. Since 10 g of glucose per liter is more than enough carbohydrate for normal bacterial growth, it seems likely that glucose stimulates EPS production. The concentration-dependent increase in EPS production was not correlated with growth, which remained almost unchanged. It has been reported previously that EPS is produced even when little or no growth occurs (5, 7). This is consistent with the proposed mechanism, namely, that slowly growing cells exhibit much slower cell wall polymer synthesis, making more isoprenoid phosphate available for EPS synthesis (28).

The distribution of sugars in EPS produced by *Lactobacillus casei* CG11 varied depending on the carbon source. This suggests that the organism may be able to produce more than one type of polymer. It has been reported previously that the EPS produced by CG11 in milk or ultrafiltrate supplemented with glucose or sucrose (10) and in BMM containing glucose or sucrose (19) differ in sugar composition. In particular, rhamnose is not present in the EPS when the strain is grown in milk or ultrafiltrate (10). In this study, rhamnose accounted for 16%

of the total component sugars in the EPS produced in BMM containing lactose and 20% of the total component sugars in the EPS produced in BMM containing glucose. It is worth mentioning that only terminal rhamnose was detected in the EPS produced by CG11 in the presence of glucose. Bryan et al. (5) reported that the rhamnose contents of the EPS produced by *Klebsiella* sp. strain K32 and *Acinetobacter calcoaceticus* depend on the carbon source available in the growth medium. The EPS produced by *Lactobacillus casei* CG11 had an original structure; galactose was not present, but 2-linked glucose was. The presence of partially methylated alditol acetates suggests that there is a repetitive sequence, as has been identified previously in other EPS (12, 15, 16, 23).

Compared with parental strain CG11, Muc$^-$ derivatives CG11-NB and CG11-ST produced very little EPS in BMM containing glucose. The levels of specific EPS production by the Muc$^-$ derivatives in the presence of glucose were distinctly lower than the level observed in the wild-type strain. A possible explanation for this is that the parental *Lactobacillus casei* strain, CG11, produces two different EPS in the presence of glucose and the production of only one is encoded by a gene(s) on the 30-kb plasmid. The results obtained with CG11 and the Muc$^-$ derivatives grown in milk ultrafiltrate and BMM containing lactose, which were not markedly different, support this explanation. On the other hand, the sugar compositions of the EPS produced by the two Muc$^-$ derivatives in BMM containing lactose or glucose were similar to each other, but different from the sugar composition of the EPS produced by strain CG11; the rhamnose contents of the EPS produced by the Muc$^-$ derivatives were markedly lower. Interestingly, fucose was detected only in the EPS produced by the two Muc$^-$ derivatives when they were grown in BMM containing lactose. This sugar has not been detected in EPS produced under any conditions by original strain CG11 or in other EPS formed by dairy lactic acid bacteria (6–8, 12, 15, 16).

The variation in the sugar compositions of the EPS produced by strain CG11 and its Muc$^-$ derivatives when they were grown with lactose could be explained by different mechanisms. One possible explanation is that the organisms produce similar polymers which have defined but different repeating units. The proportion of the individual polymers might vary depending on the carbon source and the growth stage. On the other hand, since the sugar compositions were determined by using gross mixtures of EPS, the observed variations could have resulted from different proportions of similar polymers in the strain CG11 and Muc$^-$ derivative EPS preparations analyzed; that is, strain CG11 may produce excess EPS, the synthesis of which is governed by genetic information located in the 30-kb plasmid, and the high level of EPS may mask detection of monomers, including fucose, that originate from polymers produced by both strain CG11 and its Muc$^-$ derivatives.

In conclusion, it was not possible on the basis of our data to determine definitely whether one or several EPS are produced by strain CG11, even though the hypothesis that more than one polymer is produced seems reasonable. *Lactobacillus casei* CG11 is able to produce EPS in the presence of various sugars; however, the most efficient carbon source is glucose. Plasmid-cured derivatives CG11-NB and CG11-ST have lost their mucoid character, as determined by physical observations (19), and the residual levels of EPS produced by these organisms are low (11 and 23%, respectively, of the level produced by wild-type strain CG11). To test whether the 30-kb plasmid cured from the Muc$^-$ derivatives contains complete information for EPS production, a non-EPS-producing *Lactobacillus casei* strain will be transformed with this plasmid.

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