Galactose Metabolism and Capsule Formation in a Recombinant Strain of *Streptococcus thermophilus* with a Galactose-Fermenting Phenotype

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ABSTRACT

The capsule-producing, galactose-negative *Streptococcus thermophilus* MR-1C strain was first transformed with a low-copy plasmid containing a functional *galK* gene from *Streptococcus salivarius* to generate a recombinant galactose-fermenting *Strep. thermophilus* strain named MR-AAC. Then, we compared the functional properties of *Strep. thermophilus* MR-AAC with those of the parent MR-1C strain when used as starter for fermented products and cheese. In lactose-supplemented laboratory medium, MR-AAC metabolized galactose, but only when the amount of lactose was less than 0.1% (wt/vol). After 7 h of fermentation, the medium was almost depleted of galactose. The parent strain, MR-1C, showed the same pattern, except that the concentration of galactose decreased by only 25% during the same period. It was found that, during milk fermentation and Mozzarella cheese production, the galactose-fermenting phenotype was not expressed by MR-AAC and this strain expelled galactose into the medium at a level similar to the parent MR-1C strain. In milk and in lactose-supplemented medium, capsular exopolysaccharide production occurred mainly during the late exponential phase and the stationary growth phase with similar kinetics between MR-1C and MR-AAC.

Key words: capsular exopolysaccharide, galactose metabolism, cheese, *Streptococcus thermophilus*

INTRODUCTION

Streptococcus thermophilus, a gram-positive lactic acid bacterium, is used extensively with lactobacilli in thermophilic starter cultures for the manufacture of fermented foods, such as yogurt, and Swiss- and Italian-type cooked cheeses. Most *Strep. thermophilus* strains

are unable to metabolize galactose (**Gal**[−]; Hutkins and Morris, 1987; Mora et al., 2002). The galactose moiety generated by lactose hydrolysis in growing cells is released into the medium by LacS permease, a galactose/lactose antiport system that facilitates lactose entry (Poolman et al., 1989). This metabolic defect is due, in part, to the production of insufficient levels of galactokinase, the product of the *galK* gene and a key enzyme of the Leloir pathway (Vaillancourt et al., 2002). Recently, Vaillancourt et al. (2004) demonstrated that improved galactokinase activity could be achieved by transforming the industrial strain *Strep. thermophilus* SMQ-301 with a plasmid carrying a functional *galK* gene from *Streptococcus salivarius*. Indeed, the recombinant *Strep. thermophilus* strain SMQ-301K01 acquired the ability to grow on galactose (**Gal**⁺) as a sole carbon source. However, *Strep. thermophilus* SMQ-301K01 still expelled galactose into the medium during milk fermentation. This wild-type strain and its Gal⁺ derivative do not produce any exopolysaccharides (Tremblay and Moineau, 1999).

Exopolysaccharides are produced and secreted by many lactic acid bacteria, including some strains of *Strep. thermophilus*. These polymers may be assembled in a capsular structure (capsular exopolysaccharides; **CPS**) that is tightly associated with the cell surface or they may be secreted into the growth medium (**EPS**; Broadbent et al., 2003). In situ production of exopolysaccharides by ropy (EPS⁺) *Strep. thermophilus* strains contributes to the texture of fermented dairy products. Indeed, ropy EPS⁺ *Strep. thermophilus* strains reduce syneresis, enhance firmness, and improve the mouthfeel of yogurt (de Vuyst and Degeest, 1999) and sour cream (Adapa and Schmidt, 1998). The CPS-producing (CPS⁺) *Strep. thermophilus* strains can be used advantageously as starters in Mozzarella cheese production to increase moisture retention and improve meltability (Mistry and Anderson, 1993; Perry et al., 1997) without affecting whey viscosity, given that this type of exopolysaccharide is retained within the curd (Petersen et al., 2000). *Streptococcus thermophilus* MR-1C is a Gal[−]

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CPS⁺ strain that can be used for cheese production (Broadbent et al., 2001). Cells of the MR-1C strain are surrounded by a CPS capsule of about 3 μm that is made of repeating monomers of galactose, rhamnose, and fucose at a ratio of 5:2:1 (Low et al., 1998).

The aim of the project was to produce a recombinant Gal⁺ strain by introducing a plasmid carrying a functional *galK* gene from *Strep. salivarius* into the Gal⁻ *Strep. thermophilus* MR-1C strain, and to compare the functional properties of the recombinant strain with those of the parent *Strep. thermophilus* MR-1C strain when used as a starter for fermented dairy products such as cheese.

MATERIALS AND METHODS

Bacterial Strains

Streptococcus thermophilus MR-1C was a gift from J. R. Broadbent (Western Dairy Center, Utah State University, Logan) and *Lactobacillus delbrueckii* ssp. *bulgaricus* L210R, a Gal⁻ nonropy strain, was supplied by D. St-Gelais (Lambole et al., 2003). The bacterial strains were stored frozen at -80°C in milk-based medium made of 12% (wt/vol) reconstituted low-heat skim milk powder (Agropur, Granby, Quebec, Canada) containing 5% (wt/vol) sucrose. Frozen cultures of *Strep. thermophilus* were routinely transferred to M17 broth (Terzaghi and Sandine, 1975) supplemented with 0.5% (wt/vol) lactose (**LM17**) for the wild-type strains and with 0.5% (wt/vol) galactose (**GM17**) for the Gal⁺ recombinant strain MR-AAC (see below) and grown at 40°C . *Lactobacillus delbrueckii* ssp. *bulgaricus* L210R was grown in acidic de Man, Rogosa, and Sharpe (MRS) broth at 37°C (de Man et al., 1960). All chemicals and media were obtained from Fisher Scientific (Nepean, Ontario, Canada), unless otherwise specified. Before each experiment, cell density was adjusted so that the inoculated media would give an absorbance of about 0.05 at 600 nm (**OD**₆₀₀). An aliquot of milk was diluted in PBS (10 mM sodium phosphate, 150 mM NaCl, pH 7.5) containing 50 mM EDTA before OD₆₀₀ measurement (Kouomegne et al., 1984).

Transformation

Streptococcus thermophilus MR-1C was transformed by electroporation with the plasmid pTKRL2TK (Vailancourt et al., 2004), which carries the *Strep. salivarius* *galK* gene, as described by Buckley et al. (1999). After electroporation, cells were spread on LM17 agar-plates containing erythromycin (Sigma-Aldrich, St. Louis, MO) at 5 $\mu\text{g}/\text{mL}$ and resistant colonies were purified on GM17 agar plates containing erythromycin. Plasmid was extracted from 10 mL of overnight culture using the

QIAprep Spin Miniprep Kit (Qiagen Inc., Mississauga, Ontario, Canada). The supplier's instructions were modified to include incubation with mutanolysin (500 IU/mL, Sigma-Aldrich) for 1 h at 37°C before lysis. Polymerase chain reaction amplifications were conducted using *Taq* DNA polymerase (GE Healthcare, Baie d'Urfé, Quebec, Canada). After initial heating at 94°C for 2 min, 30 amplification cycles were applied (30 s at 94°C , 30 s at 58°C , and 1 min at 72°C , with a final incubation of 5 min at 72°C). Amplicons were analyzed on agarose gel. Forward (5'-AACGTCGTGCTGAATGTGAC-3') and reverse (5'-GAATTGATCCGGTGGATGAC-3') primers were selected to overlap the vector and insert.

Fermentation Conditions in Liquid Media

Small-scale static fermentations were carried out at 40°C without pH control for up to 24 h in reconstituted milk (10%, wt/vol), and in M17 supplemented with sugar at 0.5% (wt/vol). The media were inoculated at 1% (vol/vol) with an overnight bacterial culture grown in the same medium. Growth rate was monitored in milk by recording the pH, and in M17 by measuring OD₆₀₀. The generation time (*g*) was given by the equation $g = \ln 2 / \mu$, where μ was the growth rate.

Cheese Making

Milk for laboratory-scale cheeses was prepared by mixing skim milk and 40% (wt/vol) cream, each of which had been heated at 63°C for 30 min, to reach a protein-to-fat ratio of 1.3. Protein and fat contents were determined using a MilkoScan FT 120 (Foss, Brampton, Ontario, Canada). Milk was transferred to 12 cm $\times$ 12 cm vats (144 mL/vat) that were processed simultaneously during each experiment. The vats were heated to 37°C and inoculated with 5.4×10^8 *Strep. thermophilus* cells and 1.3×10^8 *L. delbrueckii* ssp. *bulgaricus* L210R cells/mL of cheesemilk. Two starter cultures were prepared, one containing *Strep. thermophilus* MR-1C and *L. delbrueckii* ssp. *bulgaricus* L210R (MR-1C starter), and the other containing *Strep. thermophilus* MR-AAC and *L. delbrueckii* ssp. *bulgaricus* L210R (MR-AAC starter). Two vats were inoculated with each starter culture. The inoculated batches of milk were incubated for about 1 h, until the pH dropped by 0.04 pH units. Then, 0.01 mL/L of Maxiren double-strength rennet (DSM Food Specialties, Eagleville, PA) was added to each batch of milk. After 30 min at 37°C , the cheeses were cut into 1 cm³ pieces. The temperature was increased to 40°C and the vats were left without agitation for 10 min and then agitated (60 rpm) until the whey pH reached 6.0. Whey was drained off and the cheeses transferred to

plastic bags and mixed with 50 mL of water at 58°C. The cheeses were stretched by hand for 1 min and then drained. To complete wheying off, the cheeses were transferred into 50-mL sterile Falcon tubes and centrifuged at $10,000 \times g$ for 10 min at 30°C. The resulting cheeses were stored at 4°C. The moisture content of cheese was analyzed using the oven-drying method (AOAC, 1995). Aliquots of the cheeses were taken for analysis on d 1, 15, and 30. To perform *Strep. thermophilus* cell counts, the cheeses were homogenized for 3 min in 100 mL in a Stomacher 400 Lab Blender (Seward Medical, London, UK; Purvis et al., 1987), and then serially diluted in sterile peptone water (0.05%, wt/vol) and spread on LM17 agar (MR-1C) and LM17 agar containing erythromycin (MR-AAC). The mean number of cells per bacterial chain was 18, as evaluated by microscopic observation. To evaluate cheese browning, small plastic cups (diameter of 2 cm) were filled with 2 g of curd, placed in plastic bags, sealed, and heated in boiling water for 30, 60, and 90 min. The color variations between curds were measured with a Chroma meter CR-300 (Konica Minolta, Montreal, Quebec, Canada) using the Hunter scale (Lab), in which L = lightness (white = 100 and black = 0), a = redness-greenness, and b = yellowness-blueness.

Sugar and Organic Acid Profiles

Sugars and organic acids were quantified as described by St-Gelais et al. (1991). Aliquots of 5 mL of fermented medium were withdrawn and mixed with 2.5 mL of H_2SO_4 (0.02 M). The mixture was heated for 30 min at 85°C, centrifuged at $3,000 \times g$ for 15 min at 4°C, and filtered on a 0.45- μ m filter before injection onto the column. Cheeses (2.5 g) were diluted with 5 mL of H_2SO_4 (0.02 M) and homogenized in an Ultra-Turrax homogenizer (Fisher Scientific) for 2 min. The suspension was centrifuged and filtered before injection into the column. The solution was injected into an Ion 300 column (VWR International, Montreal, Quebec, Canada) mounted on a Dionex DX-500 HPLC system (Dionex Canada Ltd., Oakville, Ontario, Canada) and eluted at 40°C with H_2SO_4 (0.02 M) at a flow rate of 0.5 mL/min. The detection devices were a Shodex RI-101 Refractive Index detector for sugar and an AD20 UV detector for organic acids (Canadian Life Science Inc., Peterborough, Ontario, Canada).

Capsular Exopolysaccharide Assay

The amount of CPS on the surface of the cells was determined using a lectin binding assay carried out as described before (Robitaille et al., 2006) using 2 fluorescein isothiocyanate (FITC)-conjugated lectins (Vec-

tor Laboratories Canada Inc., Burlington, Ontario, Canada); namely, peanut agglutinin (PNA) isolated from *Arachis hypogaea* (FITC-PNA) and wheat germ agglutinin (WGA) from *Tricicum vulgaris* (FITC-WGA). Briefly, bacterial cells were collected, washed, and suspended at an absorbance at 600 nm of 1.0 in PBS containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.05% (wt/vol) Tween-20, and 4 μ g/mL of FITC-lectin. After a 30-min incubation at room temperature, cells were collected, washed, and suspended at an OD_{600} of 0.5 to record fluorescence intensity (excitation and emission wavelength: 485 and 530 nm) using an FL500 microplate fluorescence reader (BioTek Instruments Inc., Winooski, VT). The presence of CPS on the bacterial cell surface was estimated by the relative fluorescence at 530 nm (intensity in arbitrary units) detected for a constant amount of bacteria (OD_{600} of 1) using FITC-PNA as a probe (FITC-PNA relative fluorescence). Any increase in FITC-PNA relative fluorescence reflects a greater deposition of CPS on the cell surface and a thicker capsular structure (Robitaille et al., 2006). As a control, bacterial cells were probed with FITC-WGA, which binds to the peptidoglycan layer of the cell wall of gram-positive bacteria (Sizemore et al., 1990). The FITC-PNA relative fluorescence was then recorded after correction for the amount of bacterial cells (FITC-WGA relative fluorescence), which is related to the quantity of bacterial cells in the medium assuming that the amount of WGA-reactive species per cell on the peptidoglycan layer of the cell wall did not vary appreciably during growth.

Statistical Analyses

The experiments were repeated independently 3 times, and statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The galactose-negative, capsule-producing strain *Strep. thermophilus* MR-1C was transformed with the plasmid pTKRL2TK (which carries the *Strep. salivarius galK* gene) to obtain a recombinant strain named MR-AAC. The presence of pTKRL2TK in MR-AAC was confirmed by PCR (data not shown). *Streptococcus thermophilus* MR-AAC grew at 40°C in M17 supplemented with lactose, sucrose, or glucose, but its growth rate was somewhat slower than that of the parent strain. The doubling time of the wild-type strain MR-1C grown at 40°C in LM17 medium was 41 ± 5 min, compared with a doubling time of 55 ± 8 min for MR-AAC. Contrary to the wild-type strain, the recombinant strain, MR-AAC, grew on GM17 supplemented with galactose as the sole

Figure 1. Sugar and organic acid contents of M17 culture media containing 5% lactose (LM17) during fermentation at 40°C. The LM17 medium was inoculated with *Streptococcus thermophilus* MR-1C (open symbols) and MR-AAC (filled symbols). Galactose = $\circ$ —, $\square$ —; lactose = $\square$ —, $\square$ —; and lactic acid = $\triangle$ —, $\triangle$ — contents in g/L; absorbance (optical density) at 600 nm (OD_{600} ; $\diamond$ —, $\diamond$ —).

Figure 2. Sugar and organic acid contents of milk during fermentation at 40°C. Reconstituted milk (10% total solids) was inoculated with *Streptococcus thermophilus* MR-1C (open symbols) or MR-AAC (filled symbols). Concentrations of galactose ($\circ$ —, $\square$ —), glucose ($\square$ —, $\square$ —), lactic acid ($\triangle$ —, $\triangle$ —), and citric acid ($\diamond$ —, $\diamond$ —) in g/L; lactic acid/galactose molar ratio [$\times$ — (MR-1C); $\times$ — (MR-AAC)].

carbon source and its doubling time was 100 min. This doubling time is 2 times that reported for strain SMQ-301K01, which was obtained from the galactose-negative strain SMQ-301 by transformation with pTKRL2TK (Vaillancourt et al., 2004). The reason for this difference is unknown, although it could be because of strain-to-strain variations or perhaps the metabolic burden associated with CPS production by MR-1C. The plasmid pTKRL2TK was very stable in strain MR-1C, as evidenced by the fact that the Gal⁺ phenotype of strain MR-AAC was retained in more than 96% of the tested colonies after 10 subcultivations on LM17. It was, in fact, more stable than some native plasmids found in other *Strep. thermophilus* strains (Girard and Moineau, 2007).

The fate of galactose and lactose were monitored during growth in LM17 medium. As shown in Figure 1, the recombinant MR-AAC strain released free galactose into the medium when lactose was available, but to a lesser extent than the wild-type strain MR-1C. Galactose content reached a maximum of 1.6 g/L in MR-1C-fermented medium and 1.2 g/L in MR-AAC-fermented medium when the lactose concentration dropped below 1 g/L. After this peak, galactose content in MR-AAC-fermented medium decreased rapidly to undetectable levels after about 7 h of fermentation. The galactose content in MR-1C-fermented medium was still greater than 1 g/L after the same fermentation period. Thus, the presence of the extrachromosomal *galK* gene in MR-AAC reduced galactose expulsion and stimulated the metabolism of galactose to lactic acid as soon as the medium was depleted of lactose. Interestingly, the recombinant strain MR-AAC displayed the type C galactose fermentation profile, which is 1 of 4 galactose fermentation profiles reported in the literature (De Vin et

al., 2005). A similar sugar profile has been described for Gal⁺ recombinant *Strep. thermophilus* SMQ301-K01 in LM17 (Vaillancourt et al., 2004). By contrast, the wild-type strain MR-1C had a type B galactose fermentation profile in LM17; this profile is typical for a Gal⁻ *Strep. thermophilus* strain and involves a slow decrease in galactose level when lactose is depleted due to the basal activity of galactokinase.

The MR-1C and MR-AAC strains produced lactic acid at similar rates until the lactose concentration fell below 1 g/L (Figure 1). Production of lactic acid by MR-AAC was increased in lactose-depleted conditions relative to MR-1C, reflecting increased biomass production. Indeed, cell density reached 1.7 OD_{600} for MR-1C grown in LM17 for 24 h compared with 2.1 OD_{600} for MR-AAC. Thus, even though strain MR-AAC was unable to entirely prevent galactose expulsion during growth on lactose, it expressed a Gal⁺ phenotype in lactose-restricted LM17 medium.

A study was carried out to evaluate the ability of the *Strep. thermophilus* MR-1C and MR-AAC strains to metabolize galactose during milk fermentation. The galactose and organic acid production curves for MR-1C and MR-AAC during milk fermentation at 40°C are presented in Figure 2. During fermentation, the production of lactic acid and galactose gradually increased and reached a plateau after about 16 h. During the 24-h fermentation period, milk pH decreased from 6.6 to 4.6. Lactic acid and galactose secretion in the fermented milks exhibited similar trends throughout the incubation period for the 2 strains. To evaluate the fermentation profile associated with MR-AAC, the lactic acid/galactose molar ratios were determined; if the strain cannot metabolize galactose, 2 mol of lactic acid and 1 mol of galactose would be released into the medium per

Table 1. Sugar and organic acid contents of Mozzarella-type cheese produced with *Streptococcus thermophilus* MR-1C and the recombinant Gal⁺ MR-AAC as starters in combination with *Lactobacillus delbrueckii* ssp. *bulgaricus* L210R, after 1, 15, and 30 d of storage at 4°C

Item	MR-1C starter			MR-AAC starter			SEM
	1 d old	15 d old	30 d old	1 d old	15 d old	30 d old	
Lactose (g/kg)	9.0 ^a	0.7 ^b	0.0 ^b	7.3 ^a	0.9 ^b	0.0 ^b	0.5
Galactose (g/kg)	8.0 ^b	12.6 ^a	13.5 ^a	7.9 ^b	12.6 ^a	13.3 ^a	0.6
Lactic acid (g/kg)	8.0 ^b	16.4 ^a	18.3 ^a	8.4 ^b	17.1 ^a	18.2 ^a	0.6
Molar ratio ¹	2.02 ^b	2.59 ^a	2.71 ^a	2.11 ^b	2.68 ^a	2.76 ^a	0.13

^{a,b}Values with different superscripts within a row are significantly different ($P < 0.05$).

¹Molar ratio = lactic acid:galactose.

mole of metabolized lactose. The lactic acid/galactose molar ratio increased from 1.82 to 1.96 for MR-1C and reached 2.05 for MR-AAC ($P > 0.1$). These results indicate that both strains secreted almost all the galactose into milk during the fermentation process. Thus, the Gal⁺ phenotype conferred by the plasmid pTKRL2TK did not prevent galactose expulsion during milk fermentation. This may be explained by the fact that lactose is a better substrate than galactose and that the LacS permease, which operates as a galactose/lactose antiport system in *Strep. thermophilus*, is very efficient at expelling galactose into the surrounding medium to pick up lactose (Gunnewijk and Poolman, 2000).

Considering that the MR-AAC strain starts metabolizing galactose when the lactose content is low (Figure 1), a series of experiments was carried out to verify whether MR-AAC, when used as a starter, could efficiently reduce galactose content in Mozzarella cheese during maturation. The resulting cheese yields and moisture contents were $10.00 \pm 0.16\%$ (wt/vol) and $52.81 \pm 0.27\%$ (wt/wt), respectively, for the MR-1C starter and $10.05 \pm 0.16\%$ (wt/vol) and $53.68 \pm 0.26\%$ (wt/wt), respectively, for the MR-AAC starter ($P > 0.1$). In both cases, *Strep. thermophilus* bacterial counts in the cheeses reached 5×10^9 cells/mL after 1 d and did not fall below 10^9 cells/mL within 30 d. The results of sugar analyses of the cheeses during maturation at 4°C are presented in Table 1. Residual lactose in the water phase dropped below 0.2% within 15 d in both types of cheese. There was virtually no lactose in the cheeses at 30 d. The galactose level increased significantly ($P < 0.05$) from d 1 to 30 in a similar fashion for cheeses made with MR-1C or MR-AAC starters ($P > 0.1$), ultimately reaching 13 g/kg. Concomitant production of lactic acid was detected for both starters. Although galactose levels remained high during storage, the increase in lactic acid/galactose molar ratios from 2 to >2.7 ($P < 0.05$) indicated that some galactose was metabolized by both starters. The differences between cheeses made with the MR-1C starter and the MR-AAC starter were not significant ($P > 0.1$). Therefore, it appears that

MR-AAC did not metabolize galactose more efficiently than MR-1C during cheese maturation. This could be attributable to low metabolic activity in bacteria during cheese maturation, as reflected in the small amount of lactic acid produced between d 15 and 30.

Because it is already well recognized that a high galactose content results in excessive cheese browning during baking (Johnson and Olson, 1985; Matzdorf et al., 1994; Mukherjee and Hutkins, 1994), we tested whether the recombinant strain had an effect on the browning of the experimental Mozzarella cheeses. Cheese browning after 30 d of maturation was similar for cheeses made with the MR-1C starter and the MR-AAC starter ($P > 0.01$; data not shown). This was expected because the galactose contents were similar after 30 d of maturation (Table 1).

Exopolysaccharides are synthesized from activated nucleotide sugars, particularly uridine-5'-diphosphate (UDP)-glucose and UDP-galactose. These UDP-sugars can be formed from either the glucose or the galactose moiety of lactose using enzymes of the Leloir pathway. Phosphoglucosyltransferase is the enzyme that links the Leloir pathway with glycolysis, allowing the interconversion of glucose-6-phosphate and glucose-1-phosphate and allowing glucose utilization for UDP-glucose biosynthesis. Data derived for the phosphoglucosyltransferase-negative strain showed that the Leloir pathway might be responsible for the production of exopolysaccharides in lactose-growing *Strep. thermophilus*, even though the strain was Gal⁻ (Levander and Radstrom, 2001). We speculated that the restoration of galactokinase activity by inserting an exogenous *galK* gene in *Strep. thermophilus* could improve exopolysaccharide production.

A fluorescence-based lectin-binding assay was used to compare the amount of CPS produced by MR-1C and by the recombinant derivative Gal⁺ MR-AAC in milk and in LM17 media. The FITC-PNA relative fluorescence values that reflect capsule thickness are presented in Figure 3 along with the FITC-WGA relative fluorescence values, which served as fluorescence controls. In milk, the FITC-PNA relative fluorescence val-

Figure 3. Bacterial capsular exopolysaccharide production during the fermentation process as estimated from the relative fluorescence of bacterial cells after probing with fluorescein isothiocyanate-conjugated peanut agglutinin. Fermentation was carried out at 40°C in reconstituted milk (10% solids) inoculated with *Streptococcus thermophilus* MR-1C (—□—) and MR-AAC (—■—), and in LM17 inoculated with *Strep. thermophilus* MR-1C (—△—) and MR-AAC (—▲—). As a control, fluorescein isothiocyanate-conjugated wheat germ agglutinin was used to probe the bacterial cell wall during milk fermentation with MR-1C (—○—) or MR-AAC (—●—) as starters. Fermentation was tracked in milk based on pH changes (---x---) and in LM17 based on absorbance (optical density) at 600 nm (OD_{600} ; ---+---).

ues for MR-1C and MR-AAC increased steadily from 6 to 18 h, pointing to gradual deposition of CPS on the capsular structure at the end of the exponential growth phase and during the stationary growth phase; the rate and extent of CPS deposition was similar for the 2 strains ($P > 0.1$). As already observed in experiments on galactose secretion (Figure 2), the high lactose content in milk media probably inhibited the generation of galactose-1-phosphate from galactose, as well as inhibiting the use of galactose-1-phosphate for UDP-galactose biosynthesis.

It was previously shown that lactose content in LM17 decreases rapidly, making galactose available for metabolic purposes during late exponential and stationary growth phases (Figure 1). As shown by FITC-PNA relative fluorescence values for MR-1C and MR-AAC grown in LM17 (Figure 3), the gradual deposition of CPS on the capsular structure of *Strep. thermophilus* MR-1C and MR-1AAC strains occurred during the late exponential and stationary growth phases, but to a lesser extent than in milk, as expected from previous studies (Hassan et al., 2001; Robitaille et al., 2006). The rate and extent of CPS deposition was similar for the 2 strains ($P > 0.1$; Figure 3). This suggests that the increased production of galactose-1-phosphate from galactose in the MR-AAC strain due to galactose metabolism did not affect CPS production. When MR-AAC is affected by lactose privation, galactose resulting from the hydrolysis of lactose was most likely used as an energy source.

CONCLUSIONS

In this study, the low-copy plasmid pTKRL2TK carrying the *galK* gene from *Strep. salivarius* was inserted into the CPS⁺ strain *Strep. thermophilus* MR-1C to derive a galactose-fermenting recombinant named MR-AAC. This phenotype was observed primarily in lactose-free or depleted medium in the late exponential growth phase and the stationary growth phase. The presence of the plasmid had a limited effect on galactose metabolism by MR-AAC during growth in milk and during cheese storage; it had a limited effect on capsule production.

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