Detection and Quantification of Capsular Exopolysaccharides from *Streptococcus thermophilus* Using Lectin Probes

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ABSTRACT

The aim of this work was to use fluorescently labeled lectins to develop a convenient and reliable method to determine the relative abundance of capsular polysaccharides (CPS) at the surface of *Streptococcus thermophilus* MR-1C cells. Fluorescein isothiocyanate-labeled peanut agglutinin isolated from *Arachis hypogaea* was found to interact specifically with the CPS of *Strep. thermophilus* MR-1C. This labeled lectin was then used as an effective probe to detect and quantify CPS. A fluorescence-based lectin-binding assay was successfully applied to follow the accumulation of CPS during the growth of *Strep. thermophilus* MR-1C in milk and in M17 broth supplemented with lactose. Our results showed that in both media, CPS production by *Strep. thermophilus* MR-1C began during the exponential phase of growth and continued for several hours after the culture reached the stationary growth phase.

Key words: lactic acid bacteria, exopolysaccharide, capsule, cheese

INTRODUCTION

Streptococcus thermophilus is a lactic acid bacterium (LAB) extensively used in starter cultures in combination with lactobacilli to transform milk into fermented products such as yogurt and cheese. Several strains of *Strep. thermophilus* produce extracellular polysaccharides that are made of many repeating units having at least 2 different monosaccharides (Broadbent et al., 2003; Vaningelgem et al., 2004) and are thus considered heteropolysaccharides (HePS). These polymers may be assembled as capsular polysaccharides (CPS) that are tightly associated with the cell surface, or they may be secreted into the growth medium (i.e., “slime” polysaccharides, EPS; Broadbent et al., 2003). Many yogurt manufacturers are interested in the in situ production

of HePS by *Strep. thermophilus* to avoid the use of stabilizers and texturing agents such as modified starch, carrageenan, and gelatin. Indeed, EPS⁺ strains of *Strep. thermophilus* can reduce syneresis and enhance product texture and viscosity (Broadbent et al., 2003). It has also been proposed to use CPS⁺ strains of *Strep. thermophilus* for cheese manufacture because the presence of CPS favors moisture retention, increases cheese yield, and improves melting properties without affecting whey viscosity (Petersen et al., 2000). The capsule-producing strain *Strep. thermophilus* MR-1C has been extensively studied to this end (Broadbent et al., 2001). This strain produces a capsule of about 3 μ m from the cell wall to the edge of the capsule. Its CPS is made of repeating monomers of galactose, rhamnose, and fucose in the ratio of 5:2:1 (Low et al., 1998).

The production of HePS varies greatly depending on the *Strep. thermophilus* strain and the culture conditions (de Vuyst et al., 2001). In milk, for example, the amount of EPS produced by *Strep. thermophilus* may range from 50 mg to 1.5 g per liter (Degeest et al., 2001). Although several studies on HePS synthesis have been conducted with EPS⁺ *Strep. thermophilus* strains, only a limited number of studies have been carried out on CPS⁺ strains (Dabour et al., 2006). Using confocal laser scanning microscopy (CLSM), Hassan et al. (1995, 2001) found that cells of *Strep. thermophilus* that were grown in milk produced a larger capsule than cells grown in Elliker's broth. They also suggested that the composition of the growth medium can affect capsule thickness. For example, increasing the lactose content of Elliker's broth from 0.5 to 5% or adding whey protein or CN digest produced larger capsules (Hassan et al., 2001).

The classical methods for characterizing EPS production are rather fastidious because the EPS must be extracted, purified, and chemically analyzed for each strain (Deveau and Moineau, 2003; Rimada and Abraham 2003; Goh et al., 2005; Ruas-Madiedo and de los Reyes-Gavilan, 2005). Moreover, these methods cannot be directly applied to CPS because this type of HePS is tightly associated with the bacterial cell. Thus, an efficient method for the quantitative isolation of LAB cells of capsular HePS still awaits development.

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Lectins are a class of glycoproteins that bind to mono-saccharide components of oligo- and polysaccharide structures and may represent a convenient tool to probe and quantify CPS from *Strep. thermophilus*. Lectins specifically bind to bacterial cells with respect to the type of sugars exposed at the cell surface. Lectins have already been used in several applications in microbiology, such as for the detection of gram-positive bacteria using wheat germ agglutinin (WGA) from *Tricicum vulgaris* (Sizemore et al., 1990; Holm and Jespersen, 2003), as well as for the typing of pathogens (Kellens et al., 1993). Labeled lectins in combination with CLSM or transmission electron microscopy (TEM) are also effective tools to study polysaccharides in bacterial biofilm (Neu et al., 2001) and in dairy products (Hassan et al., 2000; Dabour et al., 2005; Folkenberg et al., 2005). The aim of this work was to develop a convenient and reliable method to determine the relative abundance of CPS on bacterial cells during growth under various culture conditions using fluorescently labeled lectins.

MATERIALS AND METHODS

Bacterial Strains

Streptococcus thermophilus MR-1C, a CPS⁺ strain, was a gift from J. R. Broadbent (Western Dairy Center, Utah State University, Logan, UT). The EPS⁺ strain *Strep. thermophilus* RD534 was kindly provided by Danisco (Copenhagen, Denmark; Lévesque et al., 2005). The EPS⁻ strain *Strep. thermophilus* SMQ-301 was used as a negative control (Tremblay and Moineau, 1999). These strains were routinely grown at 40°C in M17 broth (Terzaghi and Sandine, 1975) supplemented with 0.5% lactose (LM17) and stored at -80°C in milk containing 5% sucrose.

Fermentation Conditions

Stock cultures were transferred in LM17, incubated overnight at 40°C, and propagated twice in the medium that was used for fermentation. Two media were used in this study: LM17 and reconstituted skim milk powder at 10% (wt/vol; Agropur, Québec, Canada). The media were inoculated at 1% (vol/vol) and small-scale static fermentations were carried out at 40°C without pH control. Growth in milk was monitored by measuring the pH, whereas growth in LM17 was monitored by tracking the optical density at 600 nm (OD₆₀₀).

Lectin-Binding Assay

The preliminary screening of *Strep. thermophilus* strains for binding ability was carried out with 7 different fluorescein isothiocyanate (FITC)-conjugated lectins

(Vector Laboratories Inc., Burlington, Ontario, Canada) namely, concanavalin A isolated from *Canavalia ensiformis*, *Dolichos biflorus* agglutinin isolated from *Dolichos biflorus*, peanut agglutinin (PNA) isolated from *Arachis hypogaea*, *Ricinus communis* agglutinin I isolated from *Ricinus communis*, soybean agglutinin isolated from *Glycine max*, *Ulex europaeus* agglutinin I isolated from *Ulex europaeus*, and WGA. After growth in LM17, *Strep. thermophilus* cells were collected by centrifugation at 4,000 × g for 10 min. After growth in reconstituted skim milk powder, the culture was first diluted with PBS (10 mM sodium phosphate, 150 mM NaCl, pH 8) containing 50 mM EDTA, and the pH was readjusted to 8.0 with 0.2 N NaOH to dissociate CN micelles prior to cell collection by centrifugation. *Streptococcus thermophilus* cells were washed twice with PBS (pH 7.5) and resuspended at an OD₆₀₀ of 1.0 in PBS containing 1 mM MgCl₂, 1 mM CaCl₂, and 0.05% (wt/vol) Tween-20 (PBS-MCT) and FITC-conjugated lectins at 4 µg/mL final concentration. Cells were incubated at room temperature for 30 min, centrifuged, and washed twice with PBS. Finally, the cells were resuspended in a PBS:glycerol (1:1) solution containing 5 mM *p*-phenylenediamine and observed with a Nikon Eclipse E6000 (Nikon Canada, Inc., Mississauga, ON, Canada) for epifluorescence microscopy analyses. To assess the specificity of the labeling obtained with lectins, galactose, and *N*-acetylglucosamine, the target sugars for PNA and WGA, respectively, were added at 100 mM to PBS-MCT. The quantification of CPS was performed by fluorometry. After lectin binding, the *Strep. thermophilus* cells were washed and suspended in PBS at an OD₆₀₀ of 0.5, and 250 µL of the suspension was transferred into a 96-well microplate (Falcon Microtest flat-bottomed plates, 96 wells; Fisher Scientific, Nepean, Ontario, Canada). Fluorescence intensity (excitation and emission wavelengths, 485 and 530 nm) was recorded with an FL500 microplate fluorescence reader (BioTek Instruments, Inc., Winoski, VT). To normalize the fluorescence intensity, the OD₆₀₀ was measured with an EL 808 microplate reader (BioTek Instruments, Inc.). The amount of CPS on the bacterial cells was estimated by the ratio of the fluorescence intensity of FITC-PNA detected at 530 nm per OD₆₀₀ (PNA/OD₆₀₀); the amount of WGA receptor on the cells was estimated by the ratio of the fluorescence of FITC-WGA at 530 nm per OD₆₀₀ (WGA/OD₆₀₀). As the amount of CPS was corrected for cell density, any increase in PNA/OD₆₀₀ was related to a larger amount of CPS at the cell surface and a thicker capsule. Reported data are means for duplicate samples.

Lectin-Mediated Adsorption on Agarose Beads

Streptococcus thermophilus cells resuspended in PBS-MCT (OD₆₀₀ of 1.0) were incubated with 20 µL of a

suspension of lectins immobilized on agarose beads (PNA and WGA; Sigma Aldrich Canada Ltd., Oakville, Ontario, Canada) for 30 min at room temperature under agitation. The beads were washed with PBS and the adsorption of bacterial cells on the surface of the beads was observed by microscopy.

Capsule Extraction

Streptococcus thermophilus cells were washed and suspended in PBS containing 10% TCA (wt/vol) at an OD₆₀₀ of 10. The tubes containing the cell suspensions were placed in boiling water for 5 min to solubilize the capsule (hot TCA treatment). A sample was kept at 20°C for the same period of time and used as a control. The cells were then collected by centrifugation at 4,000 × *g* for 10 min, washed twice with PBS, and tested for FITC-PNA- and FITC-WGA-binding ability as described above. The solubilized CPS obtained after treatment with hot TCA were precipitated overnight at 4°C with 2 vol of ethanol. Capsular polysaccharides were recovered by centrifugation at 28,000 × *g* for 60 min in a Beckman JA 20.1 rotor (Beckman Coulter Canada, Inc., Mississauga, ON, Canada) and dissolved in PBS. Solubilized CPS were tested for the ability to react with PNA using an agglutination test. *Streptococcus thermophilus* cell suspensions (OD₆₀₀ of 2.0) were first diluted with 1 vol of a CPS solution and incubated for 30 min at room temperature. A 0.1-vol quantity of PNA (0.02 µg/mL) was then added and the suspension was agitated for another 30 min. Agglutination was observed by optical microscopy.

Electron Microscopy

The ruthenium red staining of cells of *Strep. thermophilus* MR-1C and RD534 for TEM analyses (model 420; Philips, Amsterdam, The Netherlands) was carried out essentially as described by Dabour et al. (2005).

RESULTS AND DISCUSSION

Binding of Lectins to *Strep. thermophilus* Strains

Preliminary screenings were carried out by epifluorescence microscopy to determine the ability of 7 FITC-conjugated lectins to bind to cells of *Strep. thermophilus* MR-1C (CPS⁺), RD534 (EPS⁺), and SMQ-301 (EPS⁻). Only 2 of these lectins were capable of binding to at least one *Strep. thermophilus* strain, giving a high fluorescent signal on the cells. The WGA lectin bound to all 3 *Strep. thermophilus* strains as expected, because this lectin was previously shown to bind to the peptidoglycan layer of the cell wall of gram-positive bacteria only (Sizemore et al., 1990). Fluorescein isothiocyanate-labeled PNA was

Table 1. Quantitative lectin assay on *Strep. thermophilus* MR-1C before and after a hot TCA treatment or on MR-1C suspended in TCA without heating, and in presence of competitive sugars

Probe ¹	Treatment			Inhibitory sugar added ²
	Control	TCA, 100°C	TCA, 20°C	
FITC-PNA	100	2 ³	129	15
FITC-WGA	100	143	133	21

¹FITC-PNA = fluorescein isothiocyanate-conjugated peanut agglutinin; FITC-WGA = fluorescein isothiocyanate-conjugated wheat germ agglutinin.

²Inhibitory sugars: galactose and N-acetylglucosamine for FITC-PNA and FITC-WGA, respectively.

³Fluorescence intensities expressed as percentage relative to the control (means of duplicated, CV <5%)

the only lectin that specifically interacted with the CPS⁺ strain. When free galactose (100 mM), the known target sugar for PNA, was added to the reaction mix along with FITC-PNA, or when free N-acetylglucosamine (100 mM), the target sugar for WGA, was added along with FITC-WGA, the fluorescence intensity associated with MR-1C greatly decreased compared with the value without sugar (Table 1). These competition experiments confirmed the binding specificity of the lectins toward the targeted sugars.

Localization of the PNA Receptor

The capsular location of the PNA receptor was ascertained using PNA- and WGA-immobilized agarose. When incubated together, cells of *Strep. thermophilus* MR-1C were adsorbed on PNA-immobilized agarose (Figure 1A), indicating that this lectin bound to a reachable molecule located at the bacterial cell surface. When free galactose was added to the reaction mixture, binding was prevented (Figure 1C), as previously observed by epifluorescence microscopy (Table 1). On the other hand, there was no cell adsorption on WGA-immobilized agarose (Figure 1B), indicating that WGA did not bind to the capsule surrounding the cell wall. These data strongly suggest that PNA specifically bound to galactose-containing CPS, the constituent of the capsule, located at the cell surface of MR-1C.

Removal of the Capsule of *Strep. thermophilus* MR-1C

The removal of the bacterial capsule by sonication (Ariga et al., 1992) was tested and appeared to be inappropriate with MR-1C, as evidenced by the fluorescence signal from the lectin-binding assay. An alternative method using hot TCA was developed and successfully applied. This was clearly shown by TEM after staining

Figure 1. *Streptococcus thermophilus* MR-1C reacting with immobilized lectins on agarose beads. The bacterial cells were incubated with (A) peanut agglutinin (PNA)-immobilized agarose, (B) wheat germ agglutinin-immobilized agarose, (C) PNA-immobilized agarose in the presence of 100 mM galactose, and (D) PNA-immobilized agarose after treatment at 100°C in the presence of 10% TCA (wt/vol).

with ruthenium red, a dye widely used to visualize CPS by TEM in various bacteria, including LAB (Dabour et al., 2005). An amorphous structure surrounding MR-1C bacterial cells was clearly visible (Figure 2A). When the cells were heated in the presence of 10% TCA (wt/vol) for 5 min, the amorphous structure disappeared (Figure 2B), indicating that the capsule was removed.

Lectin-binding assays were carried out to ascertain whether the amorphous structure removed by the hot TCA treatment comprised the galactose-containing material that specifically interacted with the FITC-PNA.

Figure 2. Transmission electron microscopy of *Strep. thermophilus* MR-1C before (A) and after (B) hot TCA treatment; the arrow indicates the location of the capsular structure. The capsule was stained with ruthenium red as described by Dabour et al. (2005).

When the cells were merely heated in PBS (Figure 3A) or suspended in 10% TCA without heating (Figure 3D) and then mixed with the FITC-PNA lectin, strong fluorescence was observed. However, the fluorescence signal decreased substantially (Figure 3B) when the cells were heated in the presence of 10% TCA for 1 min prior to addition of the lectin, and disappeared completely after the hot TCA treatment for 5 min (Figure 3C). In contrast, *Strep. thermophilus* cells of neither MR-1C (Figure 4B) nor RD534 (Figure 4D) lost their binding ability to FITC-WGA after the hot TCA treatment (Figure 4). These results strongly suggest that the FITC-PNA lectin bound to the amorphous material removed by the hot TCA treatment. Quantitative measurements of the fluorescence intensity (Table 1) and the binding on PNA-immobilized agarose (Figure 1D) were in agreement with the epifluorescence microscopy observations (Figures 3 and 4). Rimada and Abraham (2003) previously demonstrated that heating greatly improved HePS recovery. Our results clearly indicated that this treatment was inadequate by itself to solubilize CPS from MR-1C. Addition of TCA to precipitate proteins within the cell structure and to acidify the solution was also necessary. This suggests that the binding of CPS at the cell surface is strong and probably electrostatics in nature. In *Escherichia coli*, the presence of a membrane protein, Wzi, is needed for capsule formation (Whitfield and Paiment, 2003) but the exact mode of linkage for the bacterial capsule is still unknown.

Peanut agglutinin is a multimeric protein that binds up to 4 galactose residues, allowing cell agglutination. We used a PNA-mediated agglutination test to further substantiate that the CPS of *Strep. thermophilus* MR-1C comprised the PNA receptor. At a high cell density, PNA mediated the agglutination of MR-1C cells (Figure 5B); there was no agglutination in the absence of PNA (Figure 5A). Furthermore, neither RD-534 nor SMQ-301 agglutinated when PNA was added to the cell suspensions (data not shown). Although PNA did not cause agglutination at a low cell density (Figure 5C), agglutination was induced when soluble CPS, which were isolated from MR-1C after the hot TCA treatment, were added to the cell suspension (Figure 5D). These results suggest that soluble CPS carrying many branched galactose (Low et al., 1998) tended to form a network cross-linked by PNA, a network that can integrate cells. The formation of the network was PNA-specific, because the addition of the supernatant obtained from the hot TCA treatment of RD534 did not induce agglutination (data not shown).

Thus, by using 7 FITC-conjugated lectins as probes, we found one lectin, PNA, that could specifically bind to cells of *Strep. thermophilus* MR-1C. Consistent with previous results, we observed that this lectin bound to

Figure 3. Epifluorescence microscopy of *Strep. thermophilus* MR-1C using fluorescein isothiocyanate-labeled peanut agglutinin (PNA) as a probe. Strain MR-1C was suspended in PBS and heated at 100°C for 5 min (A); strain MR-1C was suspended in 10% TCA (wt/vol) and heated at 100°C for 1 min (B) or for 5 min (C); and strain MR-1C was suspended in 10% TCA (wt/vol) and kept at 20°C (D) before the lectin assay.

galactose molecules that were part of the capsule located at the surface of the cells (Low et al., 1998). This was further demonstrated by removing MR-1C capsules using the hot TCA treatment. The isolated CPS could still interact with the PNA lectin, as demonstrated by the agglutination experiments. These results unequivocally showed that PNA specifically reacted with the galactose-containing CPS produced by *Strep. thermophilus* MR-1C.

CPS Quantification

The lectin PNA was used to quantify the CPS in a cell suspension. The fluorescence signal was proportional to the cell content up to one OD₆₀₀ in the presence of an excess of probe (>2 µg/mL in the reaction mixture; data not shown). Thus, the method was applied to study the accumulation of CPS during the growth of *Strep. thermophilus* MR-1C in milk and in LM17 (Figure 6). In milk, CPS production began during the exponential phase of growth and continued to accumulate on the cells, as indicated by the increase in the PNA/OD₆₀₀ ratio, although the pH was below 5.0, a pH that inhibits growth. The PNA/OD₆₀₀ ratio reached a maximum value after 18 h of fermentation. In LM17, the stationary growth phase was reached after approximately 6 h of fermentation, whereas the levels of CPS (PNA/OD₆₀₀)

continued to increase steadily up to about 18 h. In contrast, the WGA/OD₆₀₀ ratio stayed relatively constant during and after milk fermentation, as expected. The results clearly showed that, in both culture media, the production of CPS began during the exponential phase of growth and, more important, continued for several hours during the stationary phase of growth. Thus, the synthesis of CPS in *Strep. thermophilus* MR-1C was not coordinated with cell division. For many slime-producing strains of *Strep. thermophilus*, the production of EPS is growth dependent (Degeest et al., 2001), but exceptions exist for some EPS⁺ strains (Gancel and Novel, 1994). Consequently, not only is HePS biosynthesis affected by culture conditions (temperature, pH, carbon and nitrogen sources, etc.), but it is also strain dependent for the type of HePS produced and the production kinetics during fermentation. Our results also showed that milk is a better medium than LM17 for CPS production, confirming previous findings comparing milk and Elliker's broth supplemented with lactose obtained by CLSM analysis (Hassan et al., 1995, 2001).

CONCLUSIONS

The industrial interest in bacterial strains producing CPS, such as *Strep. thermophilus* MR-1C, has increased in recent years because the use of these strains for milk

Figure 4. Epifluorescence microscopy of *Strep. thermophilus* MR-1C and RD534 using fluorescein isothiocyanate-labeled wheat germ agglutinin. Strain MR-1C was suspended (A) in 10% TCA (wt/vol) at 20°C or (B) in 10% TCA (wt/vol) and heated for 5 min before the lectin assay. Strain *Strep. thermophilus* RD534 was suspended in 10% TCA (wt/vol) at 20°C (C) or heated for 5 min (D) before the lectin assay.

Figure 5. The agglutination test for *Streptococcus thermophilus* MR-1C [optical density at 600 nm (OD_{600}) = 5] in PBS (A) and in PBS containing peanut agglutinin (PNA) at 0.02 $\mu\text{g/mL}$ (B). Agglutination test for MR-1C (OD_{600} = 0.5) in PBS containing PNA at 0.02 $\mu\text{g/mL}$ (C) and in PBS containing PNA at 0.02 $\mu\text{g/mL}$ and soluble capsular polysaccharides obtained from the hot TCA treatment of MR-1C (D).

fermentation improves cheese moisture retention and cheese-melting ability (Broadbent et al., 2001). Thus, substantial efforts have been devoted to developing

methods to optimize CPS biosynthesis. However, to determine whether these optimization methods are truly efficient, a procedure is needed to measure CPS production. The direct observation using CLSM is only semi-quantitative and technically rather difficult (Hassan et al., 2001). The quantification of CPS by a phenol-sulfuric acid method is possible but requires solubilization of the capsule prior to the assay. Here, we present a reliable and convenient method using a lectin that allowed the quantification of cellular-bound CPS, applied to *Strep. thermophilus* MR-1C. Moreover, we showed that complete capsule solubilization could be obtained using a single-step procedure consisting of heating cells in the presence of 10% TCA.

Taking advantage of a specific binding to CPS, we used PNA conjugated with a fluorescent probe to quantify CPS accumulation in situ during fermentation of *Strep. thermophilus* MR-1C in milk and in a semidefined culture medium. To our knowledge, this is the first report on the quantification of CPS produced by a capsular *Strep. thermophilus* strain during growth. In principle, the same strategy can be used to quantify CPS from other LAB after selecting the appropriate lectin.

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Figure 6. Growth of *Strep. thermophilus* MR-1C at 40°C in milk, as determined by the decrease in milk pH (* - -), and in M17 broth supplemented with lactose (LM17), as measured by the absorbance at 600 nm (OD_{600} ; + + +). The production of capsular polysaccharides was estimated by the relative fluorescence of cells grown in milk ($\blacksquare$ -) and in LM17 ($\blacklozenge$ -) using fluorescein isothiocyanate (FITC)-conjugated peanut agglutinin and in milk ($\bullet$ -) using FITC-conjugated wheat germ agglutinin as a probe.

REFERENCES

- Ariga, H., T. Urashima, E. Michihata, M. Ito, N. Morizono, T. Kimura, and S. Takahashi. 1992. Extracellular polysaccharide from encapsulated *Streptococcus salivarius* subsp. *thermophilus* OR 901 isolated from commercial yogurt. *J. Food Sci.* 57:625–628.
- Broadbent, J. R., D. J. McMahon, C. J. Oberg, and D. L. Welker. 2001. Use of exopolysaccharide-producing cultures to improve the functionality of low fat cheese. *Int. Dairy J.* 11:433–439.
- Broadbent, J. R., D. J. McMahon, D. L. Welker, C. J. Oberg, and S. Moineau. 2003. Biochemistry, genetics and applications of exopolysaccharide production in *Streptococcus thermophilus*: A review. *J. Dairy Sci.* 86:407–423.
- Dabour, N., G. LaPointe, N. Benhamou, I. Fliss, and E. E. Kheadr. 2005. Application of ruthenium red and colloidal gold-labeled lectin for the visualization of bacterial exopolysaccharides in Cheddar cheese matrix using transmission electron microscopy. *Int. Dairy J.* 15:1044–1055.
- Dabour, N., E. Kheadr, N. Benhamou, I. Fliss, and G. LaPointe. 2006. Improvement of texture and structure of reduced-fat cheddar cheese by exopolysaccharide-producing Lactococci. *J. Dairy Sci.* 89:95–110.
- Degeest, B., F. Vaningelgem, and L. de Vuyst. 2001. Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. *Int. Dairy J.* 11:747–757.
- Deveau, H., and S. Moineau. 2003. Use of RFLP to characterize *Lactococcus lactis* strains producing exopolysaccharide. *J. Dairy Sci.* 86:1472–1475.
- de Vuyst, L., F. de Vin, F. Vaningelgem, and B. Degeest. 2001. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *Int. Dairy J.* 11:687–707.
- Folkenberg, D. M., P. Dejmek, A. Skriver, and R. Ipsen. 2005. Relation between sensory texture properties and exopolysaccharide distribution in set and in stirred yoghurts produced with different starter cultures. *J. Texture Stud.* 36:174–189.
- Gancel, F., and G. Novel. 1994. Exopolysaccharide production by *Streptococcus salivarius* ssp. *thermophilus* cultures. 1. Conditions of production. *J. Dairy Sci.* 77:685–688.
- Goh, K. K. T., D. R. Haisman, and H. Singh. 2005. Development of an improved procedure for isolation and purification of exopolysaccharides produced by *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Appl. Microbiol. Biotechnol.* 67:202–208.
- Hassan, A. N., J. F. Frank, M. A. Farmer, K. A. Schmidt, and S. I. Shalabi. 1995. Observation of encapsulated lactic acid bacteria using confocal scanning laser microscopy. *J. Dairy Sci.* 78:2624–2628.
- Hassan, A. N., J. F. Frank, and S. I. Shalabi. 2001. Factors affecting capsule size and production by lactic acid bacteria used as dairy starter cultures. *Int. J. Food Microbiol.* 64:199–203.
- Hassan, A. N., J. F. Frank, and K. B. Qvist. 2000. Direct observation of bacterial exopolysaccharides in dairy products using confocal scanning laser microscopy. *J. Dairy Sci.* 85:1705–1708.
- Holm, C., and L. A. Jespersen. 2003. Flow-cytometric gram-staining technique for milk-associated bacteria. *Appl. Environ. Microbiol.* 69:2857–2863.
- Kellens, J. T. C., J. A. Jacobs, W. J. Peumans, and E. E. Stobberingh. 1993. The agglutination of β -haemolytic streptococci by lectins. *J. Med. Microbiol.* 39:440–445.
- Lévesque, C., M. Duplessis, J. Labonté, S. Labrie, C. Fremaux, D. Tremblay, and S. Moineau. 2005. Genomic organization and molecular analysis of the virulent bacteriophage 2972 infecting an exopolysaccharide-producing *Streptococcus thermophilus* strain. *Appl. Environ. Microbiol.* 71:4057–4068.
- Low, D., J. A. Ahlgren, D. Horne, D. J. McMahon, C. J. Oberg, and J. R. Broadbent. 1998. Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention. *Appl. Environ. Microbiol.* 64:2147–2151.
- Neu, T. R., G. D. W. Swerhone, and J. R. Lawrence. 2001. Assessment of lectin-binding analysis for *in situ* detection of glycoconjugates in biofilm systems. *Microbiology* 147:299–313.
- Petersen, B. L., R. I. Dave, D. J. McMahon, C. J. Oberg, and J. R. Broadbent. 2000. Influence of capsular and ropy exopolysaccharide-producing *Streptococcus thermophilus* on Mozzarella cheese and cheese whey. *J. Dairy Sci.* 83:1952–1956.
- Rimada, P. S., and A. G. Abraham. 2003. Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. *Lait* 83:79–87.
- Ruas-Madiedo, P., and C. G. de los Reyes-Gavilan. 2005. Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *J. Dairy Sci.* 88:843–856.
- Sizemore, R. K., J. J. Cadwell, and A. S. Kendrick. 1990. Alternate gram staining technique using fluorescent lectin. *Appl. Environ. Microbiol.* 56:2245–2247.
- Terzaghi, B. E., and W. E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. *Appl. Microbiol.* 20:807–813.
- Tremblay, D. M., and S. Moineau. 1999. Complete genomic sequence of the lytic bacteriophage DT1 of *Streptococcus thermophilus*. *Virology* 255:63–76.
- Vaningelgem, F., M. Zamfir, F. Mozzi, T. Adriany, M. Vancanneyt, J. Swings, and L. De Vuyst. 2004. Biodiversity of exopolysaccharides produced by *Streptococcus thermophilus* strains is reflected in their production and their molecular and functional characteristics. *Appl. Environ. Microbiol.* 70:900–912.
- Whitfield, C., and A. Paiment. 2003. Biosynthesis and assembly of Group 1 capsular polysaccharides in *Escherichia coli* and related extracellular polysaccharides in other bacteria. *Carbohydrate Res.* 338:2491–2502.