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Characterization of the chemical structures and physical properties of exopolysaccharides produced by various *Streptococcus thermophilus* strains

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ABSTRACT

Exopolysaccharides (EPS) produced by some lactic acid bacteria are often used by the dairy industry to improve the rheological and physical properties of yogurt, but the relationship between their structure and functional effect is still unclear. The EPS from different species, or different strains from the same species, may differ in terms of molar mass, repeating unit structure, and EPS yield during fermentation of milk. This study aimed to characterize the detailed properties of EPS produced from 7 strains of *Streptococcus thermophilus*, which is one of the key cultures used for yogurt manufacture. Milk was fermented with strains DGCC 7698, DGCC 7710, DGCC 7785, ST-10255y, St-143, STCth-9204, and ST4239. These strains were selected because they have been used in previous studies on yogurt texture, but a complete description of their EPS structural properties has not yet been reported. All strains were fermented under a similar acidification rate by adjusting the level of supplementation with peptone or the inoculation level, which allowed for a comparison of EPS yields under similar growth conditions (reconstituted skim milk at 40°C). The EPS from each strain was isolated and the weight-average molar mass and *z*-average root mean square radius determined using size-exclusion chromatography multiangle laser light scattering. The monosaccharide composition of EPS was determined using gas chromatography-mass spectrometry, and repeating unit structure was determined using nuclear magnetic resonance spectroscopy. The weight-average molar mass values of EPS ranged from 0.14 to 1.61 $\times$ 10^6 g/mol. All 7 EPS samples were uncharged. The strains ST-10255y and ST4239 had EPS with the same repeating unit structure. The monosaccharide compositions of the various EPS were mainly composed of glucose and galactose, with low levels of rhamnose in the EPS isolated from DGCC 7698, and *N*-acetylgalactosamine in the EPS from DGCC 7785, ST-10255y, and ST4239. The yields of EPS (measured when fermented milks reached pH 4.6) ranged from 8.0 to 76.4 mg of glucose equivalents/kg. In addition to (free) EPS, some strains were also able to produce capsular polysaccharide (associated with the bacterial cells) when observed with negative staining technique. The results of our study will help the dairy industry to better understand the mechanism by which different strains of *Streptococcus thermophilus* affect yogurt texture.

Key words: exopolysaccharide, acid milk gel, molar mass, *Streptococcus thermophilus*

INTRODUCTION

Some lactic acid bacteria produce exopolysaccharides (**EPS**), which are high-molar mass polysaccharides, that are either excreted into the medium (called ropy or free EPS) or located on the surface of the bacterial cells (called capsular EPS). The precise function of EPS remains complex but is likely related to cell adhesion or protection (Cerning, 1990; Mende et al., 2016). Many strains of one of the key yogurt cultures, *Streptococcus thermophilus* (**ST**), produce EPS and numerous studies report that EPS production affects yogurt texture and physical properties (Tamime and Robinson, 2007). The effect of EPS production on the physical and rheological properties of cultured dairy products has been studied (Hassan et al., 2003; Purohit et al., 2009; Kristo et al., 2011; Mende et al., 2012, 2016); however, conflicting results have been reported for the effect of EPS on yogurt texture (some suggest that EPS production enhanced textural attributes, whereas other reports suggest EPS weakened yogurt texture). A better understanding of the structure-function relationships in a yogurt matrix (e.g., the effect of the specific type of EPS on gel stiffness) remains a major challenge (Jolly et al., 2002; Mende et al., 2016).

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The amount of EPS produced during the fermentation of dairy products is quite low and ranges from 20 to 500 mg/L (Mende et al., 2012). As the amount of EPS produced in situ is relatively small compared with other yogurt stabilizers, such as pectin and carrageenan (Tamime and Robinson, 2007), molecular characteristics of EPS (i.e., chemical structure, molar mass, types of linkages, and presence of charge) may have an important effect on textural properties of yogurts (Lucey, 2004; Kristo et al., 2011; Mende et al., 2016).

The EPS produced by ST are heteropolysaccharides composed of repeating units of different monosaccharides. The main types of monosaccharides are glucose, galactose, and rhamnose (Cerning, 1990). Fucose, ribose, glucuronic acid, acetylated amino sugars moieties (i.e., *N*-acetylgalactosamine), and noncarbohydrate constituents, such as phosphate and acetate, have also been reported to be constituents of some types of EPS (Jolly et al., 2002; Girard and Schaffer-Lequart, 2008; Mende et al., 2016). The presence of phosphate or acetate groups would confer negative charge to the EPS, which could explain possible electrostatic type interactions with positive patches on caseins during the fermentation of milk (Corredig et al., 2011). The presence of side chains, length of the side chains, and frequency of branching all likely influence the rheological properties of fermented dairy products, as they affect the compactness or rigidity of EPS (Duboc and Mollet, 2001; Vincent et al., 2001; Jolly et al., 2002). Types of glycosidic linkages are another factor that could influence the textural properties of EPS in fermented dairy products (Ruas-Madiedo et al., 2002a). A possible relationship between chain stiffness and types of EPS linkages has been reported (Tuinier, 1999; Tuinier et al., 1999).

Structural characterization of the repeating unit in EPS is a complex process that involves identification of the glycosyl residues, analyses of the glycosidic linkages, and absolute configuration of the constituting monosaccharides. Therefore, a combination of several analytical techniques is required (Vincent et al., 2001; Sletmoen et al., 2003). The widely used technique for the analysis of glycosyl composition is GC-MS of volatile derivatives of monosaccharides. The EPS are first hydrolyzed with methanolic HCl and the resulting sugars are derivatized to trimethylsilylated methyl glycosides, which are then separated by GC. The identification and quantification of sugars are often done using an electron-impact ionization detector (Ruas-Madiedo and de los Reyes-Gavilán, 2005). The type of glycosidic linkages were identified by per-*O*-methylation of EPS, hydrolysis, followed by reduction with sodium borodeuteride, and acetylation. The partially methylated deuterated alditol acetates obtained can be identified by GC-MS (Faber et al., 1998; Harding et al., 2003). The structure of EPS is determined by nuclear magnetic resonance (**NMR**) spectroscopy. This technique is widely used to study the conformation of molecules in solution and allows elucidation of the type of glycosidic linkages and the structure of the repeating unit (Ruas-Madiedo and de los Reyes-Gavilán, 2005).

Molar mass ($\mathbf{M_w}$) and *z*-average root mean square radius ($\mathbf{R_g}$) of EPS are 2 of the main factors contributing to physicochemical properties of polysaccharides (Wyatt, 1993). The M_w of some types of EPS has been reported to range from 10^4 to 10^6 g/mol (De Vuyst et al., 2001); R_g is a measure of polymer size, which is the average extension of a polymer chain relative to the center of gravity (Wischnewski and Richter, 2006). The effect of EPS on the viscosity of fermented milks likely depends on the M_w of the EPS (Ruas-Madiedo et al., 2002b). Grobben et al. (1997) found that at the same total EPS concentration, high-M_w EPS produced from *Lactobacillus bulgaricus* NCFB-2722 had higher intrinsic viscosity than the lower-M_w EPS produced from the same strain. Tuinier et al. (1999) have developed an equation to estimate the intrinsic viscosity (η) of an EPS molecule from its M_w and R_g, which is as follows: $\eta = (3.1 \times 10^{24} \times R_g^3)/M_w$. The method widely used for determining the M_w and R_g of polysaccharides is size exclusion chromatography (**SEC**) multi-angle laser light scattering (**MALLS**; Laws et al., 2008). With this technique, the M_w and R_g of the EPS can be simultaneously determined (Ruas-Madiedo and de los Reyes-Gavilán, 2005).

In addition to molecular characterization of EPS, it is important to know if the type of EPS produced is ropy or capsular or both. Ropy and capsular EPS may have the same structure, but capsular EPS have sometimes been reported to have lower M_w and lower quantities (yields; Ruas-Madiedo and de los Reyes-Gavilán, 2005). Techniques used for the observation of capsular EPS include negative staining using India ink to stain the background (growth medium) and capsular EPS appear as a clear zone surrounding the bacterial cells (Ferreira et al., 2002; Yang et al., 2010).

Our study aimed to characterize the detailed chemical and physical properties of EPS produced by 7 different strains of ST, which were produced during fermentation of milk. If the repeating unit structure of EPS from a strain was not previously reported, then we determined it and analyzed the M_w, R_g, yield, and possible presence of capsular EPS. All strains were individually grown at a similar rate of acidification in reconstituted milk at the same temperature (40°C) to facilitate a comparison of their yields of EPS when grown under similar conditions.