J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2017-12835

Evaluation of the yield, molar mass of exopolysaccharides, and rheological properties of gels formed during fermentation of milk by *Streptococcus thermophilus* strains St-143 and ST-10255y

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ABSTRACT

The yield and chemical structures of exopolysaccharides (EPS) produced by many strains of *Streptococcus thermophilus* have been characterized. However, the kinetics (or production profile) for EPS during milk fermentation is not clear. In this study, we investigated whether any differences existed in the yield and molar mass of EPS when milk was fermented at the same acidification rate by 2 strains of *S. thermophilus* (St-143 and ST-10255y). The type of EPS produced by these 2 strains is different. Milk samples were analyzed for EPS concentration every 30 min during a fermentation period of 270 min (final pH 4.5) by using a modified quantification method, which was faster and validated for its recovery of added EPS. Rheological properties of milks during fermentation were also analyzed using small-strain dynamic oscillatory rheology. For the determination of molar mass, EPS extracts were isolated by ultrafiltration of whey obtained during fermentation of milk to pH values 5.2, 4.9, 4.7, and 4.5, and molar mass was analyzed using size-exclusion chromatography–multi-angle laser light scattering. During fermentation, both strains appeared to start producing significant amounts of EPS after about ~150 min, which corresponded to pH ~5.3, which was close to the point of gelation. During the remainder of the fermentation process (150–270 min), the EPS concentration from strains St-143 and ST-10255y significantly increased from 30 to 72 mg/L and from 26 to 56 mg/L, respectively. The quantity of EPS recovered by our modified method was estimated to represent ~60% of the total EPS added to milk. The molar mass of EPS produced by both strains appeared to slightly decrease during fermentation. At pH 5.2, EPS from St-143 and ST-10255y had molar masses of 2.9×10^6 and 1.4×10^6 g/mol, respectively, which decreased to 1.6×10^6 and 0.8×10^6 g/mol, respectively, when the pH of milk was 4.5. Distinct differences were apparent in the rheological properties of gels fermented by the 2 strains. At the end of fermentation, St-143 fermented milk had weaker gels with storage modulus (G′) value at pH 4.6 of 26 Pa, whereas gels made with ST-10255y were stiffer with a G′ value at pH 4.6 of 82 Pa. For St-143 gels, maximum loss tangent (LT_{max}) values were higher (0.50) and occurred earlier (at a higher pH value) than the LT_{max} values (0.46) for gels from ST-10255y strain. Because the fermentation conditions were identical for both strains, the observed changes in rheological properties could be due to the differences in chemical structures and molar mass of the EPS produced by these 2 *S. thermophilus* strains.
Key words: yogurt, exopolysaccharides, rheology, *Streptococcus thermophilus*

INTRODUCTION

Streptococcus thermophilus is 1 of the 2 species of lactic acid bacteria (**LAB**) that are extensively used in fermented milk products such as yogurt. Some strains of *S. thermophilus* have the ability to synthesize large polymeric carbohydrates, called exopolysaccharides (**EPS**). The presence of significant quantities of EPS in fermented milks is indicated by a sticky or stringy appearance termed as "ropiness" when these gels are stirred (De Vuyst and Degeest, 1999; De Vuyst and De Vin, 2007). *Streptococcus thermophilus* strains can generally synthesize 2 types of EPS. One type is synthesized inside the cell, and when released from the cell, the EPS remains attached to the exterior of cell, forming a capsule; hence, it is called capsular EPS. Another type of EPS is liberated into the medium and provides a ropy or slimy characteristic; hence, this type is called ropy EPS (Low et al., 1998; Broadbent et al., 2003).

The ability of *S. thermophilus* strains to produce EPS has been studied since the 1980s. Giraffa and Bergère (1987) reported on 3 ropy strains of *S. thermophilus*, namely CNRZ 404, CNRZ 783, and CNRZ 784, that

Received March 4, 2017.
Accepted May 6, 2017.
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produced 21 to 39 mg of EPS per liter in reconstituted skim milk supplemented with additional nutrients. Another 3 ropy strains of *S. thermophilus* were reported to produce 29 to 185 mg of EPS per liter of reconstituted skim milk, and the EPS content was greatly reduced when isolated EPS was subjected to protein hydrolysis followed by dialysis (Cerning et al., 1988). At the present time, at least 60 strains of *S. thermophilus* that produce EPS have been studied during the fermentation of milk, and the yield of EPS obtained from these various strains varies markedly (8–290 mg/L) from strain to strain (Giraffa and Bergère, 1987; Cerning et al., 1988; De Vuyst et al., 2001; Broadbent et al., 2003; Pachekrepapol et al., 2017).

Different methods have been used for the isolation and quantification of EPS from various types of fermentation media. To isolate EPS from milk, removal of milk protein has been carried out by (optional) enzymatic hydrolysis of protein, trichloroacetic acid (**TCA**) precipitation, or both. To isolate EPS from the resulting supernatant, ethanol, acetone, or both have been used; ethanol is the most commonly used solvent (Ruas-Madiedo and de los Reyes-Gavilán, 2005; Leroy and De Vuyst, 2016). Different strains may produce different amounts of EPS in milk depending on the availability of nutrients and fermentation conditions; the EPS yield is also affected by the methods used for isolation and quantification of EPS (Leroy and De Vuyst, 2016). Giraffa and Bergère (1987) and Cerning et al. (1988) isolated EPS by first hydrolyzing milk protein using Pronase enzyme after the fermentation was completed, followed by cell removal via centrifugation and multi-step precipitation of EPS by ethanol. Initially, Cerning et al. (1988) determined the EPS content by the phenol sulfuric acid method. When EPS was further subjected to dialysis, the net EPS content in the extract was markedly reduced, indicating ethanol precipitation may have been insufficient to remove all the small sugars, particularly lactose, that could interfere with the estimation of the actual quantity of EPS (Cerning et al., 1988). In another study by Faber et al. (1998), the yield of EPS was determined by removing milk protein by TCA precipitation and extensive dialysis of the supernatant. The EPS content in the supernatant was determined by high-performance gel permeation chromatography, using dextran standards. De Vuyst et al. (2003) used a method consisting of protein removal by TCA precipitation, EPS isolation by acetone precipitation, and residual protein precipitation by TCA followed by re-precipitation by acetone to finally isolate EPS free of small sugars and protein. The yields of EPS reported for the *S. thermophilus* strains used by De Vuyst et al. (2003) and Vaningelgem et al. (2004) were higher in comparison to other reports.

Not many reports exist for the yield of EPS from the same strains of *S. thermophilus* quantified using different isolation methods. Rimada and Abraham (2003) compared 8 different methods for the isolation and quantification of kefiran (the type of EPS in kefir) from milk-based media and found marked differences in the kefiran yields. Yields were higher for methods that used heating as an initial step and when multiple ethanol precipitation steps were used. Trichloroacetic acid precipitation at the beginning of the isolation procedure caused an almost 50% loss in EPS yield (Rimada and Abraham, 2003). Recently, EPS from *S. thermophilus* strains St-143 and DGCC 7710 was isolated by 2 different methods. In the study by Pachekrepapol et al. (2017), milk protein was removed from the fermented milk by TCA precipitation, and EPS was isolated from the supernatant by repeated ethanol precipitation followed by removal of small sugars by dialysis. The EPS content in freeze-dried isolate produced by DGCC 7710 and St-143 strains were 77 and 66 mg glucose equivalent (**GE**)/L of the medium, respectively (Pachekrepapol et al., 2017). Mende et al. (2012) also isolated this EPS by enzymatic hydrolysis, TCA precipitation of protein followed by precipitation with acetone and dialysis. The EPS contents in their dried sample were 110 and 137 mg of GE/L for the strains St-143 and DCGG7710, respectively (Mende et al., 2012). Goh et al. (2005) evaluated 2 methods of EPS isolation from synthetic media. In one method, protein was hydrolyzed by Pronase and precipitated by TCA. The resultant supernatant was dialyzed for 48 h. In another method (Goh et al., 2005), cells were removed by centrifugation and EPS was directly precipitated by ethanol and dialyzed for 48 h. A comparison of these 2 methods revealed a marked difference in EPS yields.

Since Doco et al. (1990) first determined the molar mass of EPS isolated from *S. thermophilus* strains, at least 40 strains of this species have been investigated for the molar masses of their isolated EPS. The reported molar mass values of the different EPS isolated from *S. thermophilus* strains have varied greatly. More than 26 *S. thermophilus* strains have been reported to produce EPS with molar mass greater than 1×10^6 g/mol. At least 14 strains have been reported to produce EPS with molar mass less than 1×10^6 g/mol, whereas 10 strains have been reported to produce 2 distinct EPS fractions, one type having molar mass values larger than 1×10^6 g/mol and another fraction with molar mass values below 1×10^6 g/mol (Doco et al., 1990; Lemoine et al., 1997; Faber et al., 1998; Marshall et al., 2001; Vaningelgem et al., 2004). The molar mass determination techniques were mainly based on gel filtration chromatography, which compared the molar mass of EPS with standards (dextran) of known molar mass