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Influence of Capsular and Ropy Exopolysaccharide-Producing
Streptococcus thermophilus on Mozzarella Cheese and Cheese Whey

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ABSTRACT

We investigated the effect of capsular and ropy exopolysaccharide-producing Streptococcus thermophilus starter bacteria on Mozzarella cheese functionality and whey viscosity. Mozzarella cheeses were manufactured with Lactobacillus helveticus LH100 paired with one of four S. thermophilus strains: MR-1C, a bacterium that produces a capsular exopolysaccharide; MTC360, a strain that secretes a ropy exopolysaccharide; TAO61, a nonexopolysaccharide-producing commercial cheese starter; and DM10, a nonencapsulated, exopolysaccharide-negative mutant of strain MR-1C. As expected, cheese moisture levels were significantly higher in Mozzarella cheeses made with exopolysaccharide-positive versus exopolysaccharide-negative streptococci, and melt properties were better in the higher moisture cheeses. Whey viscosity measurements showed that unconcentrated and ultrafiltered, fivefold concentrated whey from cheeses made with S. thermophilus MTC360 were significantly more viscous than whey from cheeses made with MR-1C, TAO61, or DM10. No significant differences were noted between the viscosity of unconcentrated or concentrated whey from cheeses made with S. thermophilus MR-1C versus the industrial cheese starter TAO61. These data indicate that encapsulated, but not ropy, exopolysaccharide-producing S. thermophilus strains can be utilized to increase the moisture level of cheese and to improve the melt properties of Mozzarella cheese without adversely affecting whey viscosity.

(Abbreviation key: Cps = capsular exopolysaccharide or the ability (+) or inability (−) to produce a capsular exopolysaccharide; EPS = exopolysaccharide or the ability (+) or inability (−) to produce exopolysaccharide.

INTRODUCTION

Many strains of dairy lactic acid bacteria synthesize extracellular polysaccharides. These compounds may be tightly associated with the cell wall (i.e., capsular), or be secreted into the medium as a loose slime (i.e., ropy). The term exopolysaccharide (EPS) is commonly used to refer to both types of extracellular polysaccharide (3, 8, 14). Milk fermented with ropy EPS-producing (EPS⁺) lactic acid bacteria generally develops a more viscous texture, and EPS⁺ strains of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus are commonly used to enhance viscosity and reduce syneresis in yogurt (3, 13).

In the dairy industry, the application of EPS⁺ starter lactic acid bacteria to enhance product functionality has traditionally been focused on the manufacture of yogurt and fermented milks. Recent research by our group, however, showed that a capsule-producing (Cps⁺) starter, S. thermophilus MR-1C, can be used to significantly increase cheese moisture level and improve the functional properties of low fat Mozzarella cheese (8, 11). While the ability to enhance cheese yield (via an increased moisture content) and functionality is an attractive incentive to the application of EPS⁺ starter bacteria in Mozzarella cheese, widespread use of these bacteria for cheese making may be limited by the fact that EPS can partition into the whey and increase whey viscosity. The accumulation of EPS in cheese whey is undesirable because it will retard the efficiency of membrane processing and thereby slow whey concentration and drying processes (9, 10, 16). In addition, the presence of EPS in whey products may alter the functional properties of these goods (E. Bastian, 1999, personal communication).

Exopolysaccharide accumulation in whey is an obvious concern for cheese making applications that employ ropy EPS⁺ starters, but Cps⁺ bacteria produce an EPS
that typically is covalently anchored to the cell surface (14). Thus, it was the hypothesis of this study that Cps\(^+\) starters were less likely than ropy EPS\(^+\) strains to affect whey viscosity. To test this hypothesis, we characterized the effect of Cps\(^+\) and ropy EPS\(^+\) S. thermophilus starter bacteria on Mozzarella cheese functionality and whey viscosity.

MATERIALS AND METHODS

Bacterial Cultures and Growth Conditions

The bacteria used in this study are described in Table 1. The presence or absence of capsular EPS was determined by the Duguid stain procedure (5) with cultures grown in 9% reconstituted skim milk, and production of ropy EPS was defined by the ability of a strain to increase the viscosity of fermented skim milk (D. Romero, 2000, personal communication). The streptococci were grown at 37\(^\circ\)C in M17 broth with 0.5% lactose (15) and Lactobacillus helveticus LH100 was propagated at 37\(^\circ\)C in MRS broth (4). All bacteria were stored at 4\(^\circ\)C and maintained by biweekly transfer.

Cheese Manufacture

Part-skim Mozzarella cheeses were manufactured on three separate occasions with Lactobacillus helveticus LH100 individually paired with each of the four S. thermophilus strains listed in Table 1. Bulk starter for cheese making was prepared by three successive overnight transfers of individual strains in 500 ml of sterilized 11% reconstituted skim milk. The cheese was manufactured using 10 kg of milk for each vat as described by Fife et al. (7), except that the curd was set at 33.3\(^\circ\)C, the cook temperature was 43.3\(^\circ\)C, and the curd was milled and dry salted (2%, wt/wt).

Cheese Composition and Functionality

Each cheese was analyzed for fat, moisture, and protein by the modified Babcock, vacuum oven, and Kjeldahl methods, respectively (1, 12). The sodium chloride and calcium contents of each cheese were also determined. To measure the sodium chloride content, grated cheese was homogenized 1:20 (wt/vol) in double deionized water, and the slurry was filtered through No. 1 filter paper (Whatman International Ltd., Maidstone, England). The concentration of sodium chloride in the filtrate was then determined with a chloride analyzer (model 926 salt analyzer; Corning, Medfield, MA) as directed by the equipment supplier. The calcium content of each cheese was determined by atomic emission spectroscopy (6). Cheese pH was measured on d 1 after pressing using an Ionalyzer (model 811; Orion Research Inc., Cambridge, MA). Cheese melt was determined within 48 h of make by the method of Bogenrief and Olson (2) as modified by Fife et al. (7).

Whey Concentration

Approximately 2.5 L of whey was collected from each vat during cheese manufacture and filtered through cheesecloth to remove curd particles. A fraction (0.5 L) of each fresh whey sample was placed at 4\(^\circ\)C for starter enumeration and viscosity tests, then the remainder was concentrated fivefold using a Minitan ultrafiltration system (Millipore, Bedford, MA) with 10-kDa membranes. The pump speed was set at 7 and a constant pressure of 10,547 kg/m\(^2\) was maintained during the UF process. The whey was kept at 4\(^\circ\)C during concentration to minimize microbial growth, and the membrane was cleaned and sanitized between each sample run as directed by the equipment manufacturer. After UF, concentrated whey samples were stored at 4\(^\circ\)C until needed.

Whey Viscosity

The viscosity of unconcentrated and concentrated whey samples was determined with a Brookfield viscometer (Model DV II; Brookfield Engineering Laboratories, Stoughton, MA) with UL adapter. The speed of the spindle was set to 30 rpm and the sample temperature was maintained at 20\(^\circ\)C using a water bath. At least 20 readings were recorded at 5-s intervals for each sample.

Determination of Starter Numbers in Cheese and Whey

The numbers of L. helveticus LH100 and S. thermophilus colony-forming units in cheese and whey were

<table>
<thead>
<tr>
<th>Table 1. Bacteria used in the study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Streptococcus thermophilus MR-1C</td>
</tr>
<tr>
<td>S. thermophilus DM10</td>
</tr>
<tr>
<td>S. thermophilus MTC360</td>
</tr>
<tr>
<td>S.thermophilus TAO61</td>
</tr>
<tr>
<td>Lactobacillus helveticus LH100</td>
</tr>
</tbody>
</table>
determined by the pour plate method with Rogosa SL (Difco Laboratories, Detroit, MI) and M17 agars, respectively, with anaerobic incubation for 2 d. Cheese for microbiological sampling was collected from each vat statistically, with serially diluted in 99 ml of sterile 2% sodium citrate immediately before plating. Fresh whey samples collected during cheese manufacture were stored overnight at 4 °C before plating. Numbers of viable starter bacteria in concentrated whey samples were determined by the pour plate method immediately after UF.

Statistical Analysis

The influence of the *S. thermophilus* starter on the viscosity of unconcentrated and concentrated whey was analyzed by ANOVA. The LSD method was also performed using SAS (SAS Inst., Inc., Cary, NC) software to compare the effect of individual *S. thermophilus* strains on whey viscosity, cheese moisture content, and cheese melt.

RESULTS AND DISCUSSION

Cheese Composition and Yield

Statistical ANOVA showed that the pH and the fat, calcium, and sodium chloride contents of the cheeses were not significantly (*P* > 0.05) affected by the strain of *S. thermophilus* used in the starter blend (Table 2). The percent protein of each cheese was significantly (*P* < 0.001) affected, and this value decreased when cheese moisture level increased. Thus, this value was lowest in cheeses made with the rropy *S. thermophilus* strain MTC360 and highest in cheese made with the EPS + strain DM10 (Table 2).

The ANOVA also showed cheese moisture level and curd yield were significantly (*P* < 0.0001) affected by the *S. thermophilus* strain used in the starter pair. As expected, cheeses made with the EPS + *S. thermophilus* strains MR-1C or MTC360 contained significantly (*P* < 0.05) more moisture than cheeses made with the nonexopolysaccharide-producing (EPS −) cocci DM10 or TAO61 (Table 2). The LSD tests also showed that part-skim Mozzarella made with the rropy EPS + culture MTC360 had a significantly (*P* > 0.05) higher moisture level than cheese made with the Cps + strain MR-1C. Finally, it may be worthwhile to note that the 3% difference in the moisture level of part-skim Mozzarella made in this study with MR-1C versus TAO61 was consistent with previous observations for low fat (6% fat) Mozzarella cheese (8,11). This result suggested that the ability of the *S. thermophilus* MR-1C Cps to increase cheese moisture content did not depend on the final fat content of the cheese.

Cheese Melt Properties

The choice of *S. thermophilus* strain also had a significant (*P* < 0.0001) effect on cheese melt properties at d 2. The average melt distance of cheese made with *S. thermophilus* MTC360 (12.5 ± 0.55 cm) was significantly (*P* < 0.05) longer than that of cheeses made with MR-1C, TAO61, or DM10 (10.4 ± 0.52, 9.3 ± 0.40, and 8.8 ± 0.19, respectively). Use of MR-1C as the starter coccus gave cheese with an average melt distance that was significantly (*P* < 0.05) longer than that from cheese made with DM10, but not TAO61. Our finding that part-skim Mozzarella cheese made with capsular or

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### Table 2. Mozzarella cheese composition.

<table>
<thead>
<tr>
<th>Component</th>
<th>DM10</th>
<th>TAO61</th>
<th>MR-1C</th>
<th>MTC360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>49.88 ± 0.38</td>
<td>51.11 ± 0.42</td>
<td>53.81 ± 0.21</td>
<td>57.22 ± 1.23</td>
</tr>
<tr>
<td>Fat</td>
<td>20.25 ± 0.14</td>
<td>20.42 ± 0.08</td>
<td>20.25 ± 0.14</td>
<td>20.08 ± 0.08</td>
</tr>
<tr>
<td>Protein</td>
<td>26.16 ± 0.13</td>
<td>24.88 ± 0.74</td>
<td>24.19 ± 0.17</td>
<td>22.52 ± 0.24</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.56 ± 0.03</td>
<td>0.56 ± 0.03</td>
<td>0.53 ± 0.02</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>Salt</td>
<td>1.41 ± 0.02</td>
<td>1.44 ± 0.04</td>
<td>1.41 ± 0.01</td>
<td>1.32 ± 0.03</td>
</tr>
<tr>
<td>pH</td>
<td>5.34 ± 0.08</td>
<td>5.23 ± 0.02</td>
<td>5.28 ± 0.02</td>
<td>5.33 ± 0.02</td>
</tr>
</tbody>
</table>

1 Cheeses were manufactured using *Lactobacillus helveticus* LH100 paired with each *S. thermophilus* strain listed. Values represent the mean percentage of the total cheese composition from three replicate experiments ± SE.
ropy EPS+ *S. thermophilus* had significantly higher moisture levels and better melt properties than cheese made with EPS− cocci was in good agreement with the observations of Perry et al. (11) for low fat Mozzarella cheese.

**Whey Viscosity**

The mean viscosity of individual whey samples in centipoise are presented in Table 3. The ANOVA showed whey viscosity was significantly (P < 0.001) affected by the *S. thermophilus* used in the cheese starter pair, and LSD tests confirmed that the mean viscosity of unconcentrated whey from cheese made with the ropy EPS+ strain, MTC360, was significantly (P < 0.05) more viscous than whey from cheeses made with MR-1C, TAO61, and DM10. This result was expected because whey from cheese made with MTC360 formed distinct, stringlike (ropy) structures during packing, piling, repiling, and milling of the curd. Ropiness was not observed in whey from cheese made with any of the other starter cocci. Fivefold UF concentrated whey from MTC360 cheese was also significantly (P < 0.05) more viscous than concentrated whey from cheeses made with MR-1C, TAO61, or DM10. In addition, whey from MTC360 cheese required more time for fivefold UF concentration. The average time required to concentrate 2 L of whey from cheese made with DM10, TAO61, or MR-1C was 4 to 4.5 h, but whey from MTC360 cheese required approximately 5.5 h.

An unexpected finding from this part of the study was that the viscosity of unconcentrated whey from MR-1C and TAO61 cheese were not significantly different from one another, both were significantly (P < 0.05) more viscous than whey from cheese made with DM10. Similar results were obtained for the concentrated whey samples; wheys from MR-1C and TAO61 cheese were not significantly different from one another, but MR-1C cheese whey was significantly more viscous than that obtained from its Cps− mutant, DM10. The basis for this observation is unknown, but one possibility is that even though TAO61 is phenotypically EPS+, this strain may produce some other type of cell surface carbohydrate (8). This hypothesis is supported by results from this study and from the work of Low et al. (8), which showed cheese made with TAO61 contained more moisture than cheese made with DM10.

**Starter Numbers**

As shown in Table 4, the *S. thermophilus* strain used in the starter pair did not appear to affect the total numbers of lactobacilli and streptococci in each cheese. In contrast, unconcentrated whey samples from cheese made with TAO61 contained populations of rods and cocci that were approximately one order of magnitude higher than those found in whey from cheeses made with the other *S. thermophilus* starters. This difference was apparently lost during UF treatment as numbers of viable starter bacteria, especially *L. helveticus* LH100, were considerably reduced in concentrated whey samples. An interesting exception to this effect was noted in concentrated whey from cheese made with MTC360, which contained approximately 10-fold higher numbers of lactobacilli (Table 4). This observation suggested that accumulation of the MTC360 ropy EPS in the whey

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**Table 3.** Mean viscosity of concentrated and unconcentrated whey.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Streptococcus thermophilus1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM10</td>
<td>TAO61</td>
<td>MR-1C</td>
<td>MTC360</td>
<td></td>
</tr>
<tr>
<td>Concentrated</td>
<td>2.37 ± 0.046</td>
<td>2.51 ± 0.067</td>
<td>2.56 ± 0.059</td>
<td>3.04 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>Unconcentrated</td>
<td>1.69 ± 0.007</td>
<td>1.93 ± 0.056</td>
<td>1.97 ± 0.073</td>
<td>2.20 ± 0.074</td>
<td></td>
</tr>
</tbody>
</table>

1Collected from cheeses manufactured using *Lactobacillus helveticus* LH100 individually paired with each *S. thermophilus* strain listed. Values represent the mean centipoise ± SE.

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**Table 4.** Numbers of starter bacteria in Mozzarella cheese and whey.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Streptococcus thermophilus1</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM10</td>
<td>TAO61</td>
<td>MR-1C</td>
<td>MTC360</td>
<td></td>
</tr>
<tr>
<td>Cheese before stretch</td>
<td>1.1 × 10⁶</td>
<td>1.1 × 10⁶</td>
<td>4.2 × 10⁹</td>
<td>4.2 × 10⁹</td>
<td></td>
</tr>
<tr>
<td>Cheese after stretch</td>
<td>1.3 × 10⁶</td>
<td>2.1 × 10⁹</td>
<td>2.9 × 10⁹</td>
<td>2.2 × 10⁹</td>
<td></td>
</tr>
<tr>
<td>Unconcentrated whey</td>
<td>2.2 × 10⁷</td>
<td>1.2 × 10⁹</td>
<td>8.1 × 10⁹</td>
<td>8.7 × 10⁹</td>
<td></td>
</tr>
<tr>
<td>Concentrated whey</td>
<td>4.3 × 10⁴</td>
<td>&lt;10²</td>
<td>5.4 × 10⁴</td>
<td>&lt;10²</td>
<td>1.2 × 10⁴</td>
</tr>
</tbody>
</table>

1Cheeses were manufactured using *Lactobacillus helveticus* LH100 individually paired with each *S. thermophilus* strain listed.
may somehow protect *L. helveticus* LH100 cells during the UF process.

**CONCLUSIONS**

This study confirmed that both ropy and capsular EPS+ *S. thermophilus* can be utilized to significantly increase cheese moisture content and to improve the melt properties of part-skim Mozzarella cheese. The ropy EPS+ starter coccus MTC360 produced cheese with significantly (*P < 0.05*) higher moisture, yield, and melt properties than cheeses made with any of the other *S. thermophilus* starters, including the Cps’ strain MR-1C. Although cheese made with MTC360 contained nearly 7% more moisture than cheese made with the EPS– commercial starter TAO61, an increase in cheese moisture of this magnitude may not be desirable. For example, part-skim Mozzarella cheese made with MTC360 expelled serum during the melt test, and this cheese was also sticky, soft, and difficult to shred. In contrast, part-skim Mozzarella made with the Cps+ starter MR-1C melted and shredded in a manner that was very similar to cheese made with the commercial starter coccus TAO61.

Another important consideration in the use of EPS+ cheese starters is their potential to deleteriously affect whey processing. Unconcentrated and UF concentrated whey from cheese made with the ropy strain MTC360 was significantly (*P < 0.05*) more viscous than comparable whey samples from cheeses made with any of the other three *S. thermophilus* starters, and it also required a longer UF processing time. In contrast, the viscosities of unconcentrated or concentrated whey from MR-1C and TAO61 cheese were not significantly different, and the time required for fivefold UF concentration of these whey samples were very similar. These results indicate that Cps+, but not ropy, *S. thermophilus* can be used as Mozzarella cheese starters to enhance cheese moisture level, yield, and melt properties without deleteriously affecting whey viscosity or UF concentration time.

**ACKNOWLEDGMENTS**

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**REFERENCES**