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Use of Galactose-Fermenting *Streptococcus thermophilus* in the Manufacture of Swiss, Mozzarella, and Short-Method Cheddar Cheese

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ABSTRACT

Galactose-fermenting (galactose-positive) strains of *Streptococcus thermophilus*, alone and combined with galactose-positive and galactose-negative strains of *Lactobacillus bulgaricus*, were used as starter cultures in the manufacture of Swiss and Mozzarella cheese and were paired with *Streptococcus lactis* (also galactose-positive) in short-method Cheddar cheese manufacture. Experimental Swiss cheese made with the galactose-positive *Streptococcus thermophilus* starter alone contained a large amount of galactose (ca. 26 to 28 μmol/g of curd) 28 h after hooping compared with control Swiss (< 2 μmol/g) made with a nongalactose fermenting strain of *Streptococcus thermophilus* and a galactose-positive strain of *Lactobacillus bulgaricus*. Mozzarella and short-method Cheddar made with only galactose-positive *Streptococcus thermophilus* also contained large amounts of galactose. Swiss cheese made with a galactose-positive strain of *Streptococcus thermophilus* and a galactose-negative strain of *Lactobacillus bulgaricus* had little galactose remaining after 28 h, indicating that the *Lactobacillus* had a stimulatory effect on galactose metabolism in *Streptococcus thermophilus*. These results indicate that galactose-fermenting *Streptococcus thermophilus* may have limited potential when used as single strain starter cultures in Swiss cheese, but may be useful when combined with galactose-positive *Lactobacillus* in the manufacture of Mozzarella cheese.

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INTRODUCTION

A number of fermented dairy products, including yogurt, Swiss, Mozzarella, and other cheese varieties, are cooked or incubated at elevated temperatures and require a thermophilic lactic starter culture. Thermophilic starter microorganisms generally include strains of *Streptococcus thermophilus* and either *Lactobacillus bulgaricus* or *L. helveticus*. These microorganisms are responsible for the conversion of lactose to lactic acid and also contribute to important sensory qualities in fermented dairy products (1).

Associative growth of *S. thermophilus* and *L. bulgaricus* in milk has been described as a symbiotic relationship [(2, 7, 26, also review 17)]. The suggested role of the *Lactobacillus* species is to degrade casein, providing the nonproteolytic *S. thermophilus* with a source of peptides and amino acids (2, 19). Production of formic acid (7) or carbon dioxide (5) by the *Streptococcus* has been reported to stimulate *L. bulgaricus*.

The sugar fermentation pattern in milk products containing *S. thermophilus* and *L. bulgaricus* led Turner et al. (25) to suggest another important role of the *Lactobacillus*. Most strains of *S. thermophilus*, unlike other lactic streptococci, are unable to ferment galactose (Gal^-) and utilize only the glucose portion of lactose, releasing free galactose into the extracellular medium (10, 13, 21, 22). In a milk fermentation, such as in Swiss cheese manufacture, rates of lactose utilization and galactose accumulation in the curd are very similar (20, 25) and reflect the initially rapid growth of *S. thermophilus* (Martley, Morris, and Gilles, unpublished data). After about 8 h of incubation, lactobacilli begin to predominate and complete the fermentation by utilizing the galactose and any remaining lactose (25). In Swiss cheese made without starter *Lactobacillus*, subsequent fermentation of galactose does not occur (25), at least within the immediate 24 h

following manufacture. Turner et al. (25) concluded that a galactose-fermenting (Gal^+) *Lactobacillus* was required for the complete fermentation of lactose and for successful Swiss cheese manufacture.

The appearance of galactose in yogurt and other cheese types made with a thermophilic starter culture containing *S. thermophilus* has also been reported (4, 9, 16, 23). Tinson et al. (23) showed that short-method (SM) Cheddar cheese made with a starter culture containing *S. thermophilus* and *S. cremoris* had a 24-h galactose concentration of 33 μmol/g cheese. Similar cheese making trials by Radford and Hull (16) resulted in cheese with galactose concentrations after 24 h of .15 to .52% (8.3 to 28.9 μmol/g). Commercial yogurt and Mozzarella cheese, both made with *S. thermophilus* and *L. bulgaricus* in the starter, were reported to contain 1.5 to 2.5% (83.3 to 138.9 μmol/g) and .8% (44.4 μmol/g) galactose, respectively (9, 11). The presence of galactose in SM Cheddar and Mozzarella cheese has been implicated in browning problems when these cheeses are processed or cooked at high temperatures (14). In addition, availability of residual galactose for heterofermentative metabolism may result in carbon dioxide production and textural defects (23).

Recently, isolation of Gal^+ strains of *S. thermophilus* has been reported (21). Although other Gal^+ strains have been reported in the literature (18), the Gal^+ variants described by Thomas and Crow were derived from Gal^- parental strains previously used as commercial starters (21), and these variants were especially suitable for cheese making trials. This study was to evaluate the performance of Gal^+ strains of *S. thermophilus* used both as a single strain starter culture and paired with various strains of *L. balgaricus* in the manufacture of Swiss and Mozarella cheese and combined with *S. lactis* in SM Cheddar cheese manufacture. Of particular interest was the ability of test strains to ferment both glucose and galactose moieties of lactose.

MATERIALS AND METHODS

Microorganisms and Starter Culture Maintenance

The Gal^+ strains of *S. thermophilus*, TS2b and MCb, derived from the parental strains unable to ferment galactose (Gal^-), TS2 and MC, respectively, were obtained from T. D. Thomas [see (21) for a description of the selection procedure]. These strains were maintained in Elliker broth (6) containing .5% galactose as the sole carbohydrate source. Inoculation into the bulk starter medium (autoclaved whole milk) was made directly from broth culture, because the Gal^+ phenotype was reported to be unstable when cultures were propagated in milk (21).

Other starter cultures included *S. thermophilus* C3 (Gal^-), *L. bulgaricus* Lb (Gal^+), *L. bulgaricus* RR (Gal^-), and *S. lactis* ML3 (Gal^+) and were obtained from the culture collection of the Department of Food Science and Nutrition, University of Minnesota, St. Paul. Although Turner and Martley (24) recently suggested that Gal^+ strains of *L. bulgaricus* be reclassified as *L. helviticus*, we have continued to use, for the present, the original species name and strain designation of the *Lactobacillus* spp. in our culture collection. These cultures were maintained in sterile 11% reconstituted nonfat dry milk prior to inoculation into the bulk starter medium.

Cheese Making Procedures

Control and experimental vats of Swiss cheese (91 kg of cheese in each) were made according to the procedure described by Gilles et al. (8) with modifications. Starter culture for the control cheese contained .45% *S. thermophilus* C3 and .05% *L. bulgaricus* Lb. Experimental cultures contained .50% of either *S. thermophilus* TS2b or MCb. Additional experimental vats were made with starter cultures containing *S. thermophilus* TS2b (.45%) and *L. bulgaricus* RR (.05%), and *S. thermophilus* C3 (.45%) and *L. bulgaricus* RR (.05%). All starter cultures also contained 12 ml/454 kg milk of *Propionibacterium freudenreichii ssp. shermanii*. After cooking and stir-out the curd was allowed to mat, and the whey was drained. The matted curd was cut into 9.1 kg blocks and placed into hoops. The cheese was pressed for 2 h and then held at 25°C for 28 h prior to brining. Samples were taken from three different sections of the cheese at intervals between the hooping and brining steps and stored at −20°C until analyzed.

Mozzarella cheese was made according to traditional manufacturing procedures (15). Control vats containing 227 kg of milk were

inoculated with 1% *S. thermophilus* C3 and 1% *L. bulgaricus* Lb, whereas experimental vats (also 227 kg) were inoculated with 2% *S. thermophilus* TS2b. Additional vats were also made with *S. thermophilus* TS2b (1%) and either *L. bulgaricus* RR (1%) or *L. bulgaricus* Lb (1%). Milk was ripened for 1 h, rennet was added, and the curd was cut and cooked at 43°C. After draining, the curd was cheddared at 43°C until 5.2 pH. Slabs were stretched under hot water (77°C), and the cheese was formed, chilled, and brined. Samples were taken at intervals and stored at −20°C as before.

Short-method Cheddar cheese was made as described by Czulak et al. (3). Control and experimental vats, each containing 454 kg of milk, were inoculated with 1% *S. lactis* ML3 and either 1% *S. thermophilus* C3 or 1% TS2b. The temperature of the cheese was maintained at 43°C during cheddaring and it was milled when the titratable acidity reached .45%. Milled curd was salted at 2.0 and .75%.

All experimental and control cheeses were made in duplicate.

Sugar Analyses

Carbohydrates were extracted by mixing 5 g of cheese and 20 ml of warm 2% sodium citrate in Whirl-Pac bags for 5 min in a Lab Blender 400 Stomacher. The slurry was acidified to pH 4.6 with 12.5% trichloroacetic acid and centrifuged for 10 min at 10,000 × *g*. The supernatant was filtered, and the filtrates were neutralized with .2 N sodium hydroxide and stored at −20°C.

Lactose and galactose were assayed enzymatically using Boehringer Mannheim (Indianapolis, IN) enzyme kits (Catalog number 176303). Each sample was assayed in duplicate. Results are the averages of at least two determinations from pooled samples.

Microbiological Analysis

Recovery and enumeration of Gal^+ *S. thermophilus* from cheese was done by stomaching 10 g of grated cheese with 90 ml of warm (45°C) 2% sodium citrate solution for 5 min. Dilutions were plated on Elliker agar (6) containing .5% galactose (filter sterilized) and 40 mg/L bromcresol purple as an acid-base indicator. Colonies surrounded by a yellow zone after 48 h incubation at 45°C were considered Gal^+.

RESULTS AND DISCUSSION

Utilization of lactose and galactose during Swiss cheese manufacture by control and experimental starter cultures is shown in Figure 1. In general, carbohydrate fermentation in Swiss cheese occurs primarily during the first 24 h after pressing, and only a small portion of the lactose is normally fermented during the setting and cooking stages of manufacture. The slight drop in pH, from 6.65 in the cheese milk to 6.60 in the experimental and control curd at draining, indicated that relatively little lactose had been utilized prior to pressing. Following pressing, however, lactose utilization occurred rapidly in control and experimental cheeses. After about 4 to 6 h, lactose was utilized more slowly, as the concentration decreased from 26 μmol lactose/g of curd at the start of pressing to less than 10 μmol lactose/g of curd at 6 h after pressing.

As lactose was depleted, galactose appeared in the curd in both the control and experimental cheese. Lactose utilization and galactose accumulation occurred at near equimolar rates. Tinson et al. (22) also reported that when *S. thermophilus* was grown in lactose broth, galactose accumulated at rates comparable to that of lactose disappearance. In the control cheese galactose accumulated only for the first 8 h (after pressing) and reached a maximum concentration of about 25 μmol/g of curd. The results indicate that nearly all of the galactose potentially available from lactose hydrolysis appeared in the curd, and none was initially fermented. After 10 to 12 h, galactose utilization was initiated, and by 26 h most of the galactose had been depleted. This fermentation pattern in the control cheese (Figure 1A) made with a traditional thermophilic starter closely resembles the pattern observed previously (20, 25).

In Swiss cheese made with *S. thermophilus* TS2b (Gal^+) and without *L. bulgaricus*, (Figure 1B), initial fermentation was similar to that of the control. As lactose was depleted, galactose accumulated. However, subsequent utilization of galactose did not occur, and instead, galactose concentration increased throughout fermentation. After 28 h all of the lactose had been utilized, but the galactose concentration remained high, about 28 μmol/g of curd, and was very near the initial lactose concentration in the pressed curd. Two additional vats of Swiss cheese, made with .50% *S. thermophilus*

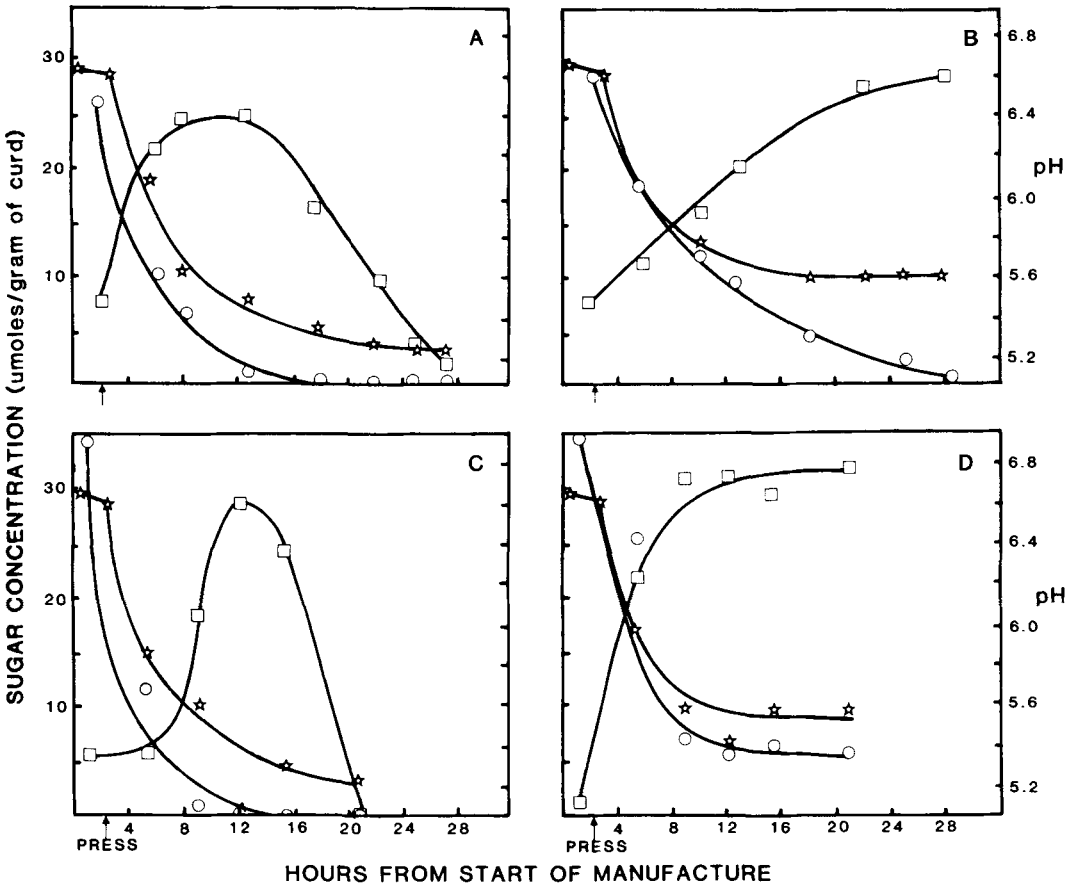

Figure 1. Utilization of lactose and galactose and changes in pH during Swiss cheese manufacture. ○, Lactose; □, galactose; ☆, pH. In A, (control) the starter culture contained .45% *Streptococcus thermophilus* C3 galactose negative (Gal^-) and .05% *Lactobacillus bulgaricus* Lb galactose positive (Gal^+). In B, the starter culture contained .5% *S. thermophilus* TS2b (Gal^+). In C, the starter culture contained .45% *S. thermophilus* TS2b (Gal^+) and .05% *L. bulgaricus* RR (Gal^-). In D, the starter culture contained .45% *S. thermophilus* C3 (Gal^-) and .05% *L. bulgaricus* RR (Gal^-).

MCb (also Gal^+), gave similar fermentation patterns with 28 h galactose concentrations of 26 μmol/g of curd. These results indicated that little, if any, galactose had been utilized by the starter organisms, and that the behavior of these Gal^+ strains of *S. thermophilus* in Swiss cheese (wthout starter lactobacilli) was similar to that of Gal^- strains of *S. thermophilus* in Swiss cheese (also without starter lactobacilli), as reported by Turner et al. (25). In addition, the final pH of the experimental cheese was 5.6, suggesting that the pH difference between the control Swiss cheese (5.2) and the experimental cheese was due exclusively to the inability of the starter bacteria to utilize and produce acid from the galactose portion of lactose. The temperature of the cheese during fermentation decreased from 52°C at the start of pressing to 30°C at 28 h after hooping and was within a range conducive to growth of *S. thermophilus* (35 to 45°C) for at least 14 h. Cheese held at 37°C during the fermentation period did not show an appreciable increase in acid development or galactose utilization compared to cheese held at 25°C (data not shown), indicating that the press room temperature had no effect on the fermentation.

In Swiss cheese made with a starter culture containing a Gal^+ strain of *S. thermophilus*, as well as a Gal^- strain of *L. bulgaricus*, sub-

sequent galactose utilization did occur (Figure 1C), resulting in a fermentation pattern similar to that of the control. The ability of *S. thermophilus* TS2b (Gal^+) to ferment the residual galactose in Swiss cheese was dependent on the presence of the *Lactobacillus* sp., even though the latter was itself Gal^-. The inability of *S. thermophilus* C3 (Gal^-) to ferment residual galactose (Figure 1D) when Gal^- lactobacilli were present further showed that Gal^+ starter organisms were required for galactose fermentation to occur in Swiss cheese.

Mozzarella Cheese

Results of the sugar fermentation in Mozzarella cheese are shown in Figure 2. Carbohydrate fermentation in Mozzarella, unlike that in Swiss cheese, occurs during the early stages of manufacture. In both control and experimental Mozzarella trials, fermentation of lactose occurred rapidly, as the lactose concentration in the curd was reduced from 95 to less than 35 μmol/g of curd during the first 5 h of manufacture. (Although some carbohydrate would be lost in the whey, the

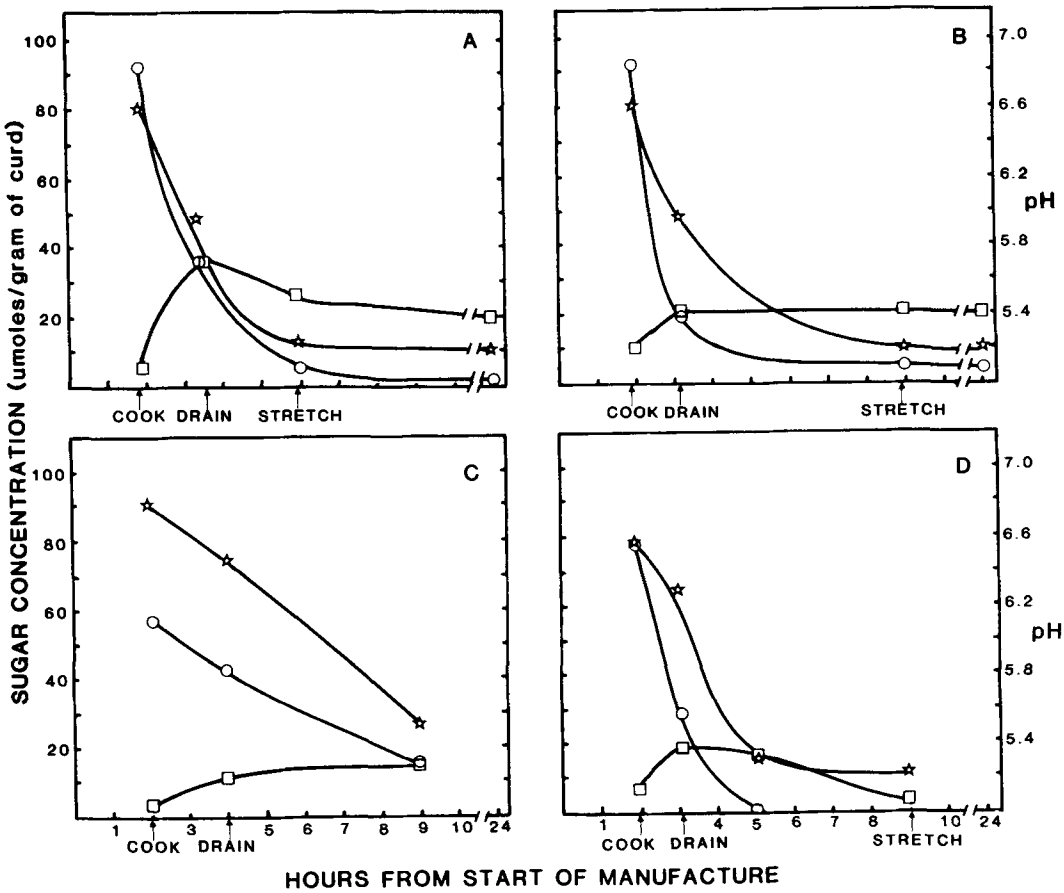

Figure 2. Utilization of lactose and galactose and changes in pH during Mozzarella cheese manufacture. ○, Lactose; □, galactose; ☆, pH. In A, (control) the starter culture contained 1% *Streptococcus thermophilus* C3 galactose negative (Gal^-) and 1% *Lactobacillus bulgaricus* Lb galactose positive (Gal^+). In B, the starter culture contained 2% *S. thermophilus* TS2b (Gal^+). In C, the starter culture contained *S. thermophilus* TS2b (Gal^+) and 1% *L. bulgaricus* RR (Gal^-). Vat C cheese did not reach pH 5.2 and was not stretched. In D, the starter culture contained 1% *S. thermophilus* TS2b (Gal^+) and 1% *L. bulgaricus* Lb (Gal^+).

drop in curd pH from 6.6 at cooking to 5.2 at stretching indicated that much of the lactose had been fermented). Galactose accumulation also occurred in Mozzarella cheese, as it did in Swiss cheese, regardless of which starter culture was used. Subsequently, galactose was partly utilized only in cheese made with a starter culture containing a Gal$^+$ *Lactobacillus* (Figure 2 A and D). Cultures containing either TS2b alone or combined with *L. bulgaricus* RR (Gal$^-$) released free galactose into the curd and had limited ability to utilize the residual galactose (Figure 2, B and C). Sugar concentrations in cheese made with both *S. thermophilus* TS2b and *L. bulgaricus* Lb (both Gal$^+$) were similar to that of the control cheese, although in both cases some galactose remained in the curd, probably because water at 77°C was applied to the curd at the stretching step, effectively ending the fermentation.

Short-Method Cheddar Cheese

Sugar utilization in SM Cheddar cheese, as in traditional Cheddar, occurs primarily during the cheddaring process. Because higher temperatures are reached during cooking and cheddaring in SM Cheddar than during traditional Cheddar manufacture, growth of *S. thermophilus* is favored and acid develops rapidly. As presented in Figure 3, lactose concentration was reduced from 100 to 15 μmol/g of curd in 2.5 h after cutting, and the set-to-salt time was about 3.75 h. Galactose accumulation occurred in both the control and experimental SM Cheddar, although at apparently slower rates than in the Swiss and Mozzarella cheeses. In both cheeses, galactose was eventually utilized to some extent, although about 12 μmol/g of curd remained after 24 h. The presence of *S. lactis* in the starter culture probably accounted for a major portion of galactose utilization. Similar final galactose concentrations in both cheeses indicated that the presence of Gal$^+$ *S. thermophilus* had little effect on the ultimate utilization of galactose. The inhibitory role of salt on *S. thermophilus* growth and activity did not appear critical, as portions of milled curd salted at .75% had identical pH and sugar concentrations as cheese salted at 2% up to 20.5 h after milling.

Two important functions of the starter culture in the manufacture of Swiss, Mozzarella, and SM Cheddar are 1) to produce sufficient lactic acid to reduce pH to desirable acidity (approximately 5.2) and 2) to deplete the available carbohydrates so that residual concentrations are very low. If these conditions are not met, product defects may result. For example, 24-h pH above 5.4 in Swiss cheese often result in overset cheese (12). In Mozzarella cheese, pH 5.2 is required to facilitate proper stretching of the curd. In addition, availability of fermentable carbohydrates, which can serve as substrates for nonstarter microorganisms, can

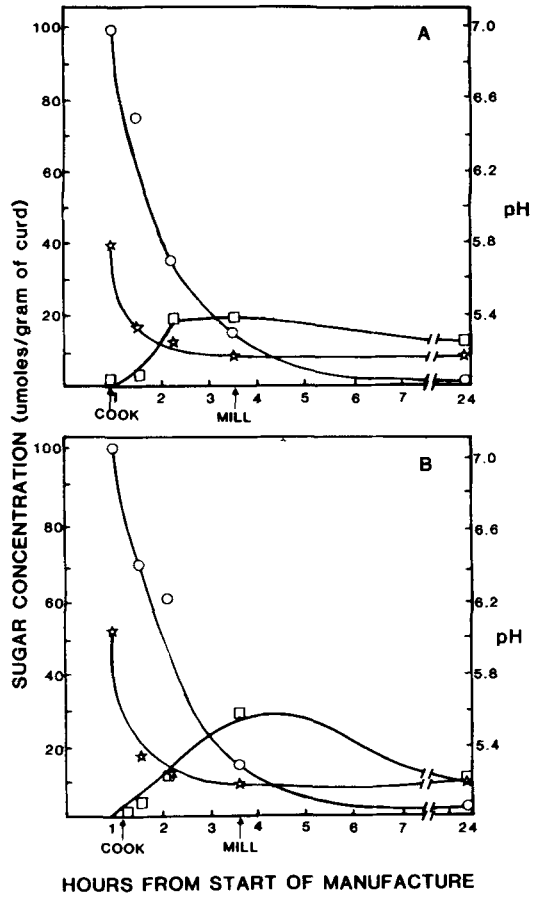

Figure 3. Utilization of lactose and galactose and changes in pH during SM Cheddar cheese manufacture. ○, Lactose; □, galactose; ☆, pH. In A, (control) the starter culture contained 1% *Streptococcus thermophilus* C3 galactose negative (Gal$^-$) and 1% *Streptococcus lactis* ML3 galactose positive (Gal$^+$). In B, the starter culture contained 1% *S. thermophilus* TS2b (Gal$^+$) and 1% *S. lactis* ML3 (Gal$^+$).

result in production of undesirable end products such as carbon dioxide (16, 23). Participation of free galactose in browning reactions, which occur during processing or cooking, is also a serious concern to the cheese industry (14).

The presence of galactose in the curd in all three experimental cheese types made with a single Gal^+ strain of *S. thermophilus* indicated that the starter culture was unable to utilize fully the galactose generated from lactose metabolism. The possibility that the starter organisms had reverted back to a Gal^- phenotype could account for these results, but the number of Gal^+ cells isolated from Mozzarella and SM Cheddar cheese was relatively large (greater than 10^8 cells/g of cheese). Because the experimental starter cultures were passed through milk only once (in the bulk starter) prior to inoculation into the cheese milk, almost 100% of Gal^+ cells were recovered from the bulk starter. After cheese making, the percent of Gal^+ cells decreased to 50 and 62% in Mozzarella and SM Cheddar, respectively.

Perhaps a more likely explanation for these results in the Swiss and Mozzarella cheeses is that the absence of starter lactobacilli limits the growth and fermentative ability of *S. thermophilus*. Although Turner et al. (25) showed that the role of starter lactobacilli was to utilize galactose in Swiss cheese, it is also known that these microorganisms stimulate *S. thermophilus* by production of amino acids and peptides (2, 26). Without the presence of the *Lactobacillus* sp., the supply of these amino acids and peptides may be limited, and thereby results in the inability of Gal^+ *S. thermophilus* to continue growth and to utilize fully residual galactose. This is supported by the observation that the addition of a Gal^- *L. bulgaricus* strain to the same Gal^+ *S. thermophilus* starter culture resulted in galactose utilization in Swiss cheese (Figure 1C). Furthermore, when grown in a complex lactose broth containing relatively large amounts of hydrolyzed protein, *S. thermophilus* TS2b was able sequentially to utilize the remaining galactose after lactose had been depleted (21). Similar results did not occur in the Mozzarella cheese (Figure 2C); the fermentation period was considerably shorter than in the Swiss cheese (Galactose utilization in Swiss did not occur until after 12 h).

The availability of Gal^+ strains of *S. thermophilus* for use in the manufacture of Swiss, Mozzarella, SM Cheddar, and other products requiring a thermophilic starter culture would be of considerable value to the industry. However, based on the results from these cheese making trials using *S. thermophilus* TS2b and MCb, a single strain Gal^+ *S. thermophilis* starter culture may be of limited value in Swiss and Mozzarella cheese manufacture. The Gal^+ strains that can metabolize both monosaccharide components of lactose, completely and simultaneously, might perform better and would be the most promising, but to our knowledge, they are presently not available. The Gal^+ strains of *S. thermophilus* used in this study combined with Gal^+ lactobacilli in Mozzarella manufacture may utilize galactose more efficiently than in traditional Mozzarella starter cultures containing Gal^- *S. thermophilus* and may be useful in this respect.

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