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Survival of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in Commercial and Experimental Yogurts

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ABSTRACT

Elliker's Lactic agar and Rogosa SL Agar, an agar medium selective for *Lactobacillus bulgaricus,* were used to determine numbers of *Streptococcus thermophilus* and *L. bulgaricus* in commercial and labortatory-manufactured yogurt. Typically, the population of viable yogurt organisms increased initially after manufacture of yogurt, and then decreased during refrigerated storage of the product. Numbers of *S. thermophilus* and *L. bulgaricus* remained above 100 million/g in plain and strawberry University of Wisconsin (UW) yogurts stored at 5°C for 60 d. Numbers of the bacteria decreased more when these yogurts were stored at 10°C, although they remained above 10 million/g, except for *S. thermophilus* in plain UW yogurt, which decreased to less than 1 million/g after 47 d of storage. Commercial custard-style plain and blueberry yogurts had *S. thermophilus* populations above 300 million/g during the 60-d storage period at both 5 and 10°C. Numbers of *L. bulgaricus* decreased from 300 million/g at 15 d after manufacture to less than 1 million/g after 42 to 56 d. Numbers of *S. thermophilus* in commercial stirred plain and raspberry yogurts remained above 1 million/g when the products were stored at 5°C for 60 d, although they decreased to less than 1 million/g after 48 to 58 d when yogurt was stored at 10°C. The *L. bulgaricus* populations decreased to less than 1 million/g after 25 to 32 d for both varieties of stirred yogurt at both storage temperatures. Presence of various fruits in commercial yogurts had little effect on survival of the yogurt bacteria. With laboratory-manufactured yogurt, different incubation temperatures and milk mixes used to make the yogurt had little effect on survival of the yogurt organisms during subsequent refrigerated storage of the product.

Yogurt is a fermented milk product made with *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* These two organisms have a symbiotic relationship during the manufacture of yogurt, with the ratio of *S. thermophilus* to *L. bulgaricus* constantly changing. *S. thermophilus* grows quickly at first, using essential amino acids produced by *L. bulgaricus*. *S. thermophilus* produces lactic acid, which lowers the pH to a more optimal level for growth of *L. bulgaricus,* along with lesser amounts of formic acid which stimulates growth of *L. bulgaricus (20)*. Growth of *S. thermophilus* then slows while *L. bulgaricus* reduces the pH even further by producing lactic acid. The pH of commercial yogurt usually is in the range of 3.7 to 4.3 (*5*). *S. thermophilus* produces some diacetyl, which gives yogurt its creamy or buttery flavor, whereas *L. bulgaricus* produces acetaldehyde which helps to give yogurt its characteristic sharp flavor (*6*). Both organisms produce lactic acid as the main fermentation product (*16*). For proper flavor development, the ratio of *S. thermophilus* to *L. bulgaricus* should be in the range of 1:1 to 3:1 (*14,19*).

The composition of yogurt is approximately the same as that of the milk from which it was made. Yogurt is an excellent source of protein, particularly when the milk mix from which it is made is fortified with nonfat dried milk (*9*).

Since 1970, yogurt has rapidly become a popular product in the U.S. Sales of yogurt have almost quadrupled between 1970 and 1980. In 1970, 171.6 million lb of yogurt were sold, with an annual per capita consumption of 0.86 lb, whereas in 1980, 588.8 million lb were sold, with an annual per capita consumption of 2.67 lb (*21*).

Yogurt also is popular in Europe, especially in Switzerland, where, in 1979, the annual per capita sales were 13.6 L (approximately 31 lb) (*3*). Some European countries have proposed or established standards for the bacterial content of yogurt. France requires that yogurt must contain at least 100 million viable *S. thermophilus* plus *L. bulgaricus* per ml within 4 d after manufacture or when it is imported (*1*). Switzerland requires that the number of viable yogurt organisms does not fall below 1 million per ml at the time of sale (*8*). Italy and Spain require that the yogurt organisms be viable and present in large numbers at the time of sale (*2,11*). A standard of 10 million viable lactobacilli per ml has been proposed in Great Britain for yogurt when it is sold (*5,18*).

An important consideration in developing and enforcing standards is information about changes that may occur in the population of *S. thermophilus* and *L. bulgaricus* during storage (usually refrigerator temperatures of 0 to 10°C) of yogurt. Davis et al. (*4*), using LAB Medium, found few changes over a 28-d period in viable counts of *S. thermophilus* and *L. bulgaricus* in yogurt that was stored at 5°C. However, at 15°C the viable counts decreased markedly after 14 d. Lusiani et al. (*10,12*) observed that all types of yogurt (including fruit yogurt) still contained more than 10 million viable lactic acid bacteria per ml after 40 d at 5°C, although the numbers decreased to less than 100,000/ml after 16 d when yogurt was held at 22°C. Glättli et al. (*8*) stored yogurt at 10 and 20°C, and found that the number of lactic acid bacteria decreased rapidly after 40 and 25 d, respectively, but numbers did not decrease to less than 1 million/ml during 80 d of storage at 5°C. Puhan et al. (*17*) found that numbers of yogurt organisms in commercial yogurts increased initially but began to decrease in less than 8 d after manufacture; numbers of yogurt bacteria had decreased by at least 60% after 20 d of storage. The same authors (*16*) indicated that viability of *S. thermophilus* and *L. bulgaricus* in yogurt was dependent upon pH. Numbers of *S. thermophilus* increased in yogurts with an initial pH greater than 4 until the pH decreased to 4.0 ± 0.05, and then the numbers diminished rapidly. Numbers of *L. bulgaricus* either remained constant or increased for the first 10 to 20 d in yogurt with an initial pH greater than 4 and then decreased. Ottogalli et al. (*13*) found that the lactic acid bacteria disappeared from fruit yogurt after 20 d at 4°C and after 15 d at 20°C.

The objective of the present study was to determine the survival of *L. bulgaricus* and *S. thermophilus* in some commercial and laboratory-prepared yogurts.

MATERIALS AND METHODS

Survival of S. thermophilus *and* L. bulgaricus *in commercial yogurt*

To determine survival of *S. thermophilus* and *L. bulgaricus* in commercial yogurt during storage, two samples each of three different unflavored commercial yogurts, University of Wisconsin (UW) yogurt, a popular custard-style yogurt and a popular stirred yogurt, were plated on Elliker's Lactic Agar (*7*) and on Rogosa SL Agar (Difco) every 7 d from the time of purchase to 60 d past the date of manufacture. Elliker's Lactic Agar gives total numbers of the viable yogurt organisms, whereas Rogosa SL Agar gives the numbers of *L. bulgaricus*. The numbers of *S. thermophilus* can then be calculated to be the difference between the numbers of *L. bulgaricus* and the total numbers of yogurt bacteria.

Along with these samples of unflavored yogurt, two samples of a fruit yogurt from each of the three manufacturers were also plated on Elliker's Lactic Agar and on Rogosa SL Agar to observe if the presence of fruit affected survival of the yogurt organisms. Fruit yogurts used were strawberry, blueberry and raspberry.

One sample of each of the six commercial yogurts was stored at 5°C, whereas the other was stored 10°C. The date of manufacture was known to be 30 d before the printed code date for the UW yogurt and was assumed to be 30 d before the code dates for the other two manufacturers.

For 2 weeks during the storage study, Rogosa SL Agar was unavailable and Modified Hansen's Yogurt Agar (*15*) was substituted as good differential counts had been obtained with this medium during preliminary experiments.

A pH determination was made and a gram strain was prepared from each sample every 7 d.

Survival of S. thermophilus *and* L. bulgaricus *in laboratory-manufactured yogurt*

To evaluate effects the milk mx (milkfat or no milkfat), temperature of incubation and storage temperature may have on survival of *S. thermophilus* and *L. bulgaricus*, various batches of yogurt were made in the laboratory with two different cultures.

One culture was derived from a popular brand of unflavored custard-style yogurt. The other culture was obtained from a carton of unflavored yogurt manufactured at the University of Wisconsin. Both cultures were transferred twice into tubes of sterile 12% reconstituted nonfat milk and checked microscopically.

Four lots of yogurt were prepared using the culture from the popular brand of custard-style yogurt. The following variations were studied: (*a*) lot 1 - skim milk with incubation at 37°C during manufacture, (*b*) lot 2 - skim milk with incubation at 43°C during manufacture, (*c*) lot 3 - whole milk with incubation at 37°C during manufacture, and (*d*) lot 4 - whole milk with incubation at 43°C during manufacture of the yogurt. The yogurt was manufactured from 2 L of pasteurized skim milk or 2 L of pasteurized whole milk, each of which received an added 6% solids in the form of nonfat dry milk (120 g NFDM/2 L of milk). The resulting milk mixes were pasteurized at 72.5 ± 1.5°C for 30 min. The milk mixes then were cooled to 45°C and inoculated with 2.5% of the culture to be tested. The inoculated milk was stirred to assure even distribution of the culture, and then was poured into 20 eight-ounce waxed cartons (10 cartons with whole milk and 10 cartons with skim milk). Five cartons of the inoculated skim milk and five of the inoculated whole milk were incubated at 37°C, representing lots 1 and 3, respectively. The other five cartons of the inoculated skim milk and five cartons of the inoculated whole milk were incubated at 43°C, representing lots 2 and 4, respectively. One carton from each lot was used to check the pH during incubation. When the pH decreased to 4.5, two cartons of each lot were placed in a refrigerator at 5°C, whereas the other two were placed in a refrigerator at 10°C. The content of cartons incubated at 37°C took 4 h and 50 min to reach pH 4.5, whereas that of cartons incubated at 43°C took 3 h and 40 min. After 18 h in the refrigerator, the pH of all lots had decreased to 4.30, and gram stains prepared from each lot indicated the presence of both *L. bulgaricus* and *S. thermophilus* in all products.

Eight cartons representing the four lots of yogurt being stored at two temperatures, were plated using Elliker's Lactic Agar and Rogosa SL Agar 36 h after manufacture and every 7 d thereafter up to 60 d.

Another four batches of yogurt were prepared using the culture obtained from yogurt manufactured at the University of Wisconsin Dairy Plant. These batches of yogurt were made using the variations and procedures just described except that the milk mixes were not pasteurized after NFDM was added. The milk mix incubated at 37°C required 5 h for its pH to decrease to 4.5, whereas at 43°C the pH decreased to 4.5 in 4 h. After 18 h in the refrigerator, the pH of all lots had dropped to 4.30. Gram stains prepared from each lot revealed the presence of both rods and cocci.

RESULTS

Survival of S. thermophilus *and* L. bulgaricus *in commercial yogurt*

The "typical" curve for populations of *L. bulgaricus* and *S. thermophilus* in yogurt showed an initial increase in numbers of the yogurt organisms during and immediately after manufacture followed by a decrease during refrigerated storage. This initial increase and subsequent decrease was accelerated at 10°C as compared to 5°C. However, the three commercial yogurts we tested differed greatly in the manner in which they demonstrated this "typical" population curve.

University of Wisconsin yogurt. Numbers of yogurt organisms in University of Wisconsin (UW) plain yogurt decreased only slightly during storage at 5°C for 60 d (data not shown). At this temperature, numbers of both *L. bulgaricus* and *S. thermophilus* remained high (above 100 million/g), whereas the pH decreased from 4.55 to

4.17. However, when duplicate samples were stored at 10°C, numbers of *L. bulgaricus* decreased by a factor of 10 over the 60-d storage period, whereas *S. thermophilus* counts decreased more rapidly to less than 1 million/g and the pH decreased to 3.95 (data not shown). Survival curves for yogurt organisms in UW strawberry yogurt changed more dramatically than they did for plain yogurt (Fig. 1). There was a rapid increase in numbers of *L. bulgaricus* from 150 million/g 4 d after manufacture to 400 million/g at 11 d after manufacture beyond which numbers decreased. *S. thermophilus* increased in population from 850 million/g 4 d after manufacture to 1.3 billion/g at 19 d after manufacture, and then the numbers decreased. The death rate for the yogurt organisms decreased markedly 10 d past the time of their maximum populations when yogurt was stored at 5°C; this was not true when yogurt was stored at 10°C. The pH decreased to 4.05 after 60 d at both storage temperatures.

The UW plain yogurt did not exhibit the dramatic initial increase in numbers of yogurt organisms found with the UW strawberry yogurt. However, the plain yogurt was not plated until 10 d after manufacture (when it became available), by which time the numbers of *L. bulgaricus* in the UW strawberry yogurt had already peaked. Also, total numbers of yogurt organisms in both varieties of UW yogurt remained high when they were stored at 5°C for 60 d, whereas their numbers decreased rapidly when the product was stored at 10°C.

Commercial custard-style yogurt. The two varieties of a popular commercial custard-style yogurt, plain and blueberry, were not available on the shelves of a local supermarket until 15 d after their manufacture. By this time, numbers of the yogurt organisms appeared to have reached their maxima as there was little further increase during subsequent storage (Fig. 2). For both varieties, yogurt stored at 10°C exhibited a slightly greater decrease in numbers of *S. thermophilus* than did yogurt stored a 5°C, although the *S. thermophilus* populations at both temperatures remained very high (greater than 300 million/g) for the entire storage period. The numbers of *L. bulgaricus,* on the other hand, decreased very rapidly 20 d after the date of manufacture for both varieties of this commercial product. The numbers of *L. bulgaricus* in the plain yogurt stored at 5°C decreased from 300 million/g at 15 d after manufacture to less than 1 million/g at 56 d after manufacture. The numbers of *L. bulgaricus* in the plain yogurt stored at 10°C and in the blueberry yogurt stored at either 5°C or 10°C, decreased to less than 1 million/g after only 42 d. All samples of the commercial custard-style yogurt remained above pH 4 for the entire storage period.

Commercial stirred yogurt. Samples of a popular commercial stirred plain yogurt were difficult to find in the supermarket; the only samples that could be located during this study were tested initially at 21 d after manufacture. At this point, the number of viable yogurt organisms present was already decreasing. Samples of stirred raspberry yogurt appeared in the supermarket 6 d after manufacture. At this point in the life of the product, numbers of *S. thermophilus* were already at their maximum, whereas the *L. bulgaricus* population was still increasing (Fig. 3). The numbers of *L. bulgaricus* reached its peak at 14 d after manufacture. At its maximum, the number of *S. thermophilus* was still almost 20 times greater than that of *L. bulgaricus.* Numbers of *S. thermophilus* started to decrease rapidly in both varieties of stirred yogurt at 25 d after manufacture when stored at 5°C and at 20 d after manufacture when stored at 10°C. The numbers of *L. bulgaricus* in stirred raspberry yogurt started to decrease rapidly after only 14 d at both storage temperatures. Numbers of both yogurt organisms decreased more rapidly when both varieties of stirred yogurt were held at 10°C rather than at 5°C.

Numbers of *S. thermophilus* in both varieties of stirred yogurt stored at 5°C remained above 4 million/g for 60 d after manufacture, whereas numbers decreased to less than 1 million/g after 58 d for the plain and after 48 d

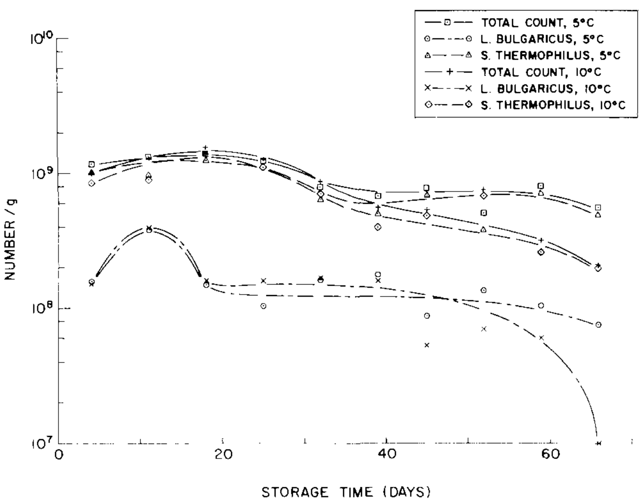

Figure 1. *Survival of* L. bulgaricus *and* S. thermophilus *in UW strawberry yogurt stored at 5 and 10°C.*

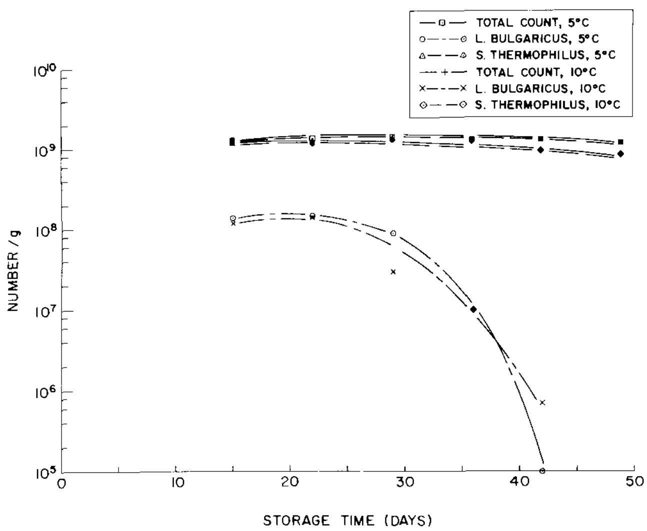

Figure 2. *Survival of* L. bulgaricus *and* S. thermophilus *in commercial custard-style yogurt when stored at 5 and 10°C.*

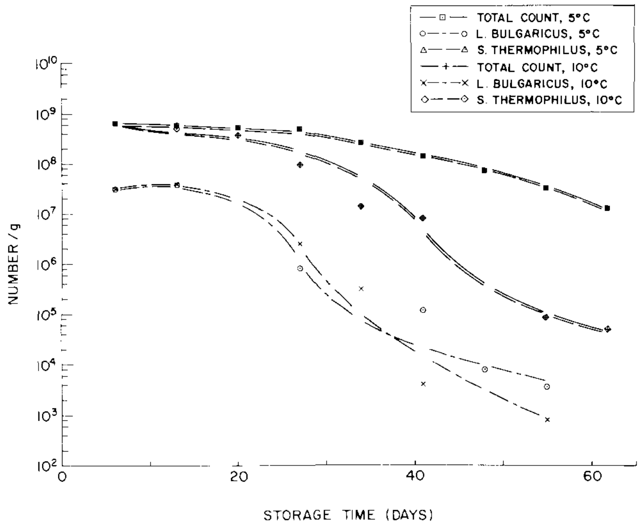

Figure 3. *Survival of* L. bulgaricus *and* S. thermophilus *in commercial stirred raspberry yogurt stored at 5 and 10°C.*

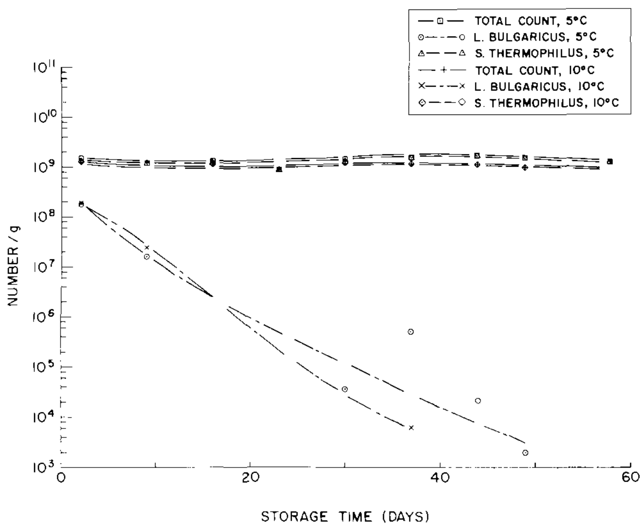

Figure 4. *Survival of* L. bulgaricus *and* S. thermophilus *in yogurt made from whole milk with a culture obtained from commercial custard-style yogurt and incubation at 37°C and then stored at 5 and 10°C.*

for the raspberry yogurt stored at 10°C. The number of *L. bulgaricus* in the stirred plain yogurt decreased to less than 1 million/g 32 d after manufacture when the product was stored at 5°C and at 25 d when it was held at 10°C. For the stirred raspberry yogurt, the number of *L. bulgaricus* decreased to less than 1 million/g after 32 and 30 d at 5 and 10°C, respectively. The pH for all samples of stirred yogurt remained above 4.1 during the entire storage period.

Laboratory-manufactured yogurt

Survival curves of *S. thermophilus* were similar for all four lots of yogurt made with each of the two cultures (one derived from a commercial custard-style yogurt and the other from University of Wisconsin yogurt). The survival curves for *L. bulgaricus* were also similar for the different lots of yogurt made in the laboratory. There was a slight increase in numbers of *S. thermophilus* initially, followed by relatively little die-off throughout the storage period. However, the numbers of *L. bulgaricus* started to decrease rapidly from the onset of the storage period (Fig. 4). The pH for all lots of yogurt decreased to between 4.13 and 4.19 for samples stored at 5°C and to between 4.01 and 4.08 for those held at 10°C.

Yogurts made with the culture from a commercial custard-style yogurt. The numbers of *S. thermophilus* remained high for the 58-d storge period at both temperatures for all four lots of yogurt (skim milk yogurt cultured at 37 and 43°C and whole milk yogurt cultured at 37 and 43°C). Initial numbers of *S. thermophilus* for all four lots ranged from 710 million to 1.3 billion/g (data not shown). Numbers at the end of the 58-d storage period ranged from 960 million to 1.5 billion/g when yogurt was stored at 5°C and from 360 million to 1.1 billion/g when it was stored at 10°C. Numbers obtained at the end of the 58-d storage period from samples held at 10°C were all lower than those from samples stored at 5°C.

The numbers of *L. bulgaricus* decreased rapidly from the initial value obtained by plating the sample 2 d after manufacture. The initial numbers of *L. bulgaricus* ranged from 150 million to 250 million/g. These numbers decreased to less than 1 million/g after approximately 22 and 15 d for yogurt manufactured from skim milk and stored at 5 and 10°C, respectively (data not shown). For yogurt manufactured from whole milk, numbers of *L. bulgaricus* decreased to less than 1 million/g after approximately 27 and 22 d when the product was stored at 5 and 10°C, respectively.

Incubation at 43°C rather than at 37°C favored growth of *L. bulgaricus*. This is shown by the ratio of *S. thermophilus* to *L. bulgaricus*. The ratio when incubation was at 37°C was 6.89:1 (average of four samples) as opposed to 5.09:1 (average of four samples) at 43°C. Also, the whole-milk mix appeared to favor growth of *L. bulgaricus*. The ratio of *S. thermophilus* to *L. bulgaricus* was 4.13:1 (average of two samples) for cultured whole milk yogurt incubated at 43°C as opposed to 6.10:1 (average of two samples) skim milk yogurt incubated at 43°C.

Numbers of *S. thermophilus* did not differ appreciably among lots of yogurt made from different milk mixes and incubated at different temperatures. However, numbers of *L. bulgaricus* decreased less rapidly in lots of yogurt made from whole milk, than in yogurt made from skim milk.

Yogurts made with the culture from UW yogurt. Numbers of *S. thermophilus* in this product corresponded to those of a typical survival curve for yogurt organisms in the product during storage, with an initial increase followed by a decrease. Initial numbers of *S. thermophilus* in lots of yogurt made with skim milk incubated at 37 and 43°C, and with whole milk incubated at 37°C, ranged from 600 million to 870 million/g and subsequently increased to between 990 million to 1.3 billion/g after 8 to 15 d of storage (data not shown). Initial numbers for the lot of yogurt made with whole milk and incubated at 43°C were between 190 and 220 million/g, and increased to between 600 and 640 million/g after 8 d of storage (data not shown). *S. thermophilus* populations in lots of yogurt incubated at 37°C began to decrease slowly 30 d after manufacture, wehreas numbers in lots of yogurt incubated at 43°C began to decrease more rapidly after only 12 d storage. The *S. thermophilus* populations in all four lots of yogurt remained above 100 million/g when the product was stored at 5°C for up to 50 d. Storage periods for yogurt held at 10°C were shortened somewhat because of mold growth on the samples. Numbers of *S. thermophilus* for skim milk and whole milk yogurts incubated at 37°C and stored at 10°C remained above 500 million/g after storage for 28 and 43 d, respectively. The *S. thermophilus* population in skim milk yogurt incubated at 43°C and stored at 10°C remained above 400 million/g after storage for 28 d, whereas the numbers of *S. thermophilus* in whole milk yogurt incubated at 43°C and stored at 10°C decreased to 17 million/g after 35 d at storage.

Numbers of *L. bulgaricus* decreased rapidly in all lots of yogurt from initial values between 190 and 460 million/g at 1 d after manufacture. The *L. bulgaricus* populations in whole milk yogurts decreased more rapidly than in skim milk yogurts. At approximately 17 d after manufacture, numbers in whole milk yogurts had decreased to below 1 million/g at both storage temperatures. The *L. bulgaricus* populations for skim milk yogurts stored at 5°C needed 31 to 34 d to decrease to less than 1 million/g, whereas those stored at 10°C needed only 26 to 30 d.

Incubation at 43°C rather than at 37°C favored growth of *L. bulgaricus*. The ratio of *S. thermophilus* to *L. bulgaricus* was 3.03:1 (average of four samples) when incubated at 37°C compared to 1.1:1 (average of four samples) when incubated at 43°C. A whole-milk mix also appeared to favor growth of *L. bulgaricus*. The UW yogurt culture incubated in whole milk at 43°C yielded a ratio of 0.45:1 (average of two samples) as opposed to 2.05:1 (average of two samples) when incubated in skim milk at 43°C.

With the UW yogurt culture, numbers of *S. thermophilus* decreased more rapidly for yogurts incubated at 43 than at 37°C. However, the *S. thermophilus* populations in all four lots of yogurt made with the UW culture remained above 100 million/g when stored at 5°C for 50 d. Numbers of *L. bulgaricus* decreased more rapidly in whole milk than in skim milk yogurt. The *L. bulgaricus* populations decreased to less than 1 million/g in whole milk yogurts after approximately 17 d, whereas skim milk yogurts were held for 26 to 34 d before population decreased to less than 1 million/g.

DISCUSSION

Commercial yogurt

Data from all commercial yogurts tested suggest a typical survival curve for the yogurt organisms. The population increased initially, reached a maximum and then decreased. Numbers of *L. bulgaricus* decreased faster than did those of *S. thermophilus* for one of the three commercial yogurts tested (numbers of each of the yogurt organisms decreased fairly equally for the other two commercial yogurts).

Presence of fruit, strawberries, blueberries or raspberries, in commercial yogurts tested, appeared to have little effect on the overall survival curves for *L. bulgaricus* and *S. thermophilus*. Shapes of the curves, maximum numbers of each organism, populations maintained for the 60-d storage period and number of days after manufacture that elapsed before the number of viable *L. bulgaricus* or *S. thermophilus* dropped below 1 million/g were very similar for the unflavored and fruit yogurts of the same manufacturer.

However, survival curves of yogurt organisms were quite different for yogurts from the three manufacturers. This suggests that manufacturing practices and various strains of starter cultures employed by the different manufacturers have a greater effect on survival of *L. bulgaricus* and *S. thermophilus* than does the presence or absence of fruit in the yogurt.

Numbers of yogurt organisms increased more during the log (or growth) phase and decreased faster during the death phase when the various yogurts were stored at 10 rather than at 5°C, although the maximum counts appeared to occur at the same time after manufacture when duplicate samples of yogurt were stored at the two temperatures.

Yogurt made in the laboratory

Numbers of *S. thermophilus* in these yogurts generally increased initially during storage and remained high (greater than 1 million/g) for their respective storage periods, whereas numbers of *L. bulgaricus* generally decreased from the beginning of the storage period. Although the numbers of viable bacteria often remained high (above 1 million/g) during storage, the *S. thermophilus* to *L. bulgaricus* ratio changed quickly in favor of *S. thermophilus*.

The incubation temperature during manufacture of yogurt influenced the initial numbers of *L. bulgaricus* and *S. thermophilus*, although it had little effect on survival of the yogurt organisms over an extended storage period. This suggests that a manufacturer could vary the incubation temperature between 37 and 43°C to achieve a desired result without affecting survival of the yogurt organisms during subsequent storage of the product. The milk mix may have some effect on survival of *L. bulgaricus* in the resultant yogurt, although the effects were not the same in all test yogurts. With laboratory-manufactured yogurts, as with commercial yogurts, numbers of yogurt organisms both increased and decreased more rapidly when products were stored at 10°C than at 5°C.

On the basis of our observations, it would be reasonable to expect yogurt to contain at least one million viable yogurt organism/g at the time of sale. None of the yogurts we tested contained less than this number when stored at 5°C for 60 d. Yogurt with less than one million viable yogurt bacteria/g would probably have been made with a slow-growing culture, heat treated after manufacture or subjected to extended temperature abuse.

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