GROWTH RATE OF COLIFORM ORGANISMS IN COTTAGE CHEESE AND RECONSTITUTED NONFAT DRY MILK

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(Received for publication November 28, 1963)

SUMMARY

The objective of this study was to determine the population trends of representative strains of Escherichia coli and Aerobacter aerogenes in cottage cheese and reconstituted nonfat dry milk (skim milk). Inoculated milk samples were stored at 32, 39, 50 and 90 F for a maximum of 130, 76, 119, and 11 days, respectively. The inoculated cheese samples were stored at 39, 50 and 55 F for 80 days. In milk at 50 F the generation time of E. coli during the logarithmic growth phase varied from 516 to 642 minutes and at 90 F the generation time varied from 33 to 39 minutes, respectively. The corresponding generation times for A. aerogenes were 540 to 648 minutes and 29 to 33 minutes. Both coliform species decreased in population at 32 and 39 F and the decrease was greater at 39 F.

In cottage cheese the population of both coliform species increased at 55 F and decreased at 39 F, but at 50 F the numbers of E. coli increased while those of A. aerogenes decreased. During the first five days at 55 F, the generation times of E. coli averaged 31.4 hours while A. aerogenes averaged 32.8 hours. At 50 F the generation time of E. coli averaged 48.2 hours.

Sanitation and keeping quality are important factors in consumer acceptance of food commodities. Between 1950 and 1960 the per capita consumption of cottage cheese increased from 3.1 to 4.8 lb (13) in the United States. The per capita sales of skim milk increased from 14.7 to 23.1 lb (12).

Initial coliform populations of < 1 per ml or g in processed fluid milk and cottage cheese have shown significant increases during storage and movement through sales outlets. The normal growth range of the coliforms is 50 to 113 F, but they have been known to survive or even increase in milk products stored at 35 to 45 F (2, 4, 6, 7, 15, 16, 17). The purpose of this study was to determine the population trends and generation times of typical strains of Escherichia coli and Aerobacter aerogenes in cottage cheese and reconstituted nonfat dry milk (skim milk) at various refrigeration temperatures. The growth pattern and generation time of the organisms were also determined at the optimum temperature of 90 F. The presence of coliform bacteria in cottage cheese has been reported by many workers, but the rate of increase of these organisms in cheese has not been investigated thoroughly. Overcast and Britton (14) manufactured cottage cheese which contained coliform counts of 10 or less per g after storage for 11 days at 40 F.

The generation time of coliform organisms grown in broth media has been determined by several workers. Barber (3), using a single cell isolation technique, found the maximum rate of growth of Bacillus (Escherichia) coli occurred at 99.5 F with a minimum generation time of 17.2 min. Additional generation times were 25 min at 90 F and 12.5 hr. at 48 to 50 F. Jennison (10) determined that the generation times were 25 min at 90 F and 12.5 hr at 26 min and that of one A. aerogenes strain was 21 min when grown in nutrient broth at 90 F. Ingram (9) reported that the generation times of E. coli (strain K-12) grown in a broth medium were 21 min at 90 to 111 F, 30 min at 90 F and 20 hr at 50 F.

EXPERIMENTAL PROCEDURE

Preparation of Milk.

Low heat non-fat dry milk was reconstituted to 9% serum solids and steamed for one hour at 212 F on each of three successive days. Between heatings, the milk was stored at room temperature. After the last heat treatment, the samples were adjusted to pH 6.9 to 6.7 with a 10% solution of sterile trisodium phosphate and stored at 39 F until used. Sample bottles containing 100 ml of the sterile reconstituted non-fat milk were tempered at 32, 39, 50 and 90 F for 24 hr before the initial coliform inoculum was added.

Manufacture of Cottage Cheese.

The cottage cheese was manufactured by the short set method, cooked to 135 F and held in the whey at 135 F for 20 min to reduce the number of psychrophiles in the curd to non-detectable levels. The curd was washed three times with water containing...
15 ppm of a hypochlorite solution. During and after the manufacturing process the cheese curd was handled carefully to minimize contamination.

A creaming mixture containing 10.5% milk fat was steamed at 212°F for one hour. Sodium chloride equal to 3.5% by weight was then added. A sufficient quantity of the creaming mixture was blended with the cheese curd to give a concentration of 3% milk fat in the cottage cheese.

Isolation and Identification of Coliform Organisms.

Coliform bacteria were isolated from raw bulk tank milk initially on Violet Red Bile (VRB) agar plates incubated at 90°F. The isolates were purified and classified according to the characteristics described by Breed et al. (5).

Propagation of Coliform Organisms.

Four representative strains of E. coli and three representative strains of A. aerogenes were selected and maintained on Tryptone Glucose Yeast (TGY) agar slants. Cultures were activated in sterile reconstituted non-fat milk, and transferred daily. An 18-hr culture was diluted with buffered sterile water to give the desired initial population and added to the reconstituted non-fat milk or creaming mixture. Initial coliform populations of 10 or less per ml were added to the milk stored at 50 and 90°F; whereas, 1500 to 2000 organisms per ml were added to the milk samples stored at 32 and 39°F. Fifteen hundred coliforms per ml added to the creaming mixture gave recoverable initial populations in the cottage cheese of about 250 per g. The reduction was attributed to the dilution of the creaming mixture with the cheese curd and to change in environment.

Preparation of Cottage Cheese Samples.

After the creaming mixture was mixed with the curd, four lots of creamed cottage cheese were prepared and inoculated with coliform organisms. Each trial included three groups of cheese samples: (a) control, (b) inoculated with E. coli and (c) inoculated with A. aerogenes. The creamed cottage cheese was measured into sterilized bottles and incubated at 39, 50 and 55°F.

Bacteriological Analyses of Samples.

Equal volumes of cottage cheese and 2% sodium citrate solution were weighed into a sterilized Waring blender jar and mixed for three minutes at slow speed. Appropriate dilutions of cheese or milk were plated on VRB agar and incubated at 95 and 90°F, respectively, for 24 hr. The higher incubation temperature was used to minimize psychrophilic growth which may have developed in the cottage cheese. Procedures outlined in Standard Methods (1) were used in determining all bacterial counts. The coliform count of control and inoculated samples of the reconstituted non-fat milk and cottage cheese was determined at appropriate intervals, selected according to the storage temperature of the samples.

Calculation of Generation Times.

The generation times were calculated according to the following formula:

\[
\text{generation time} = \frac{(T_e - T_i) \times \log 2}{\log b - \log B}
\]

where:

- \(T_e\) = interval of time (minutes or hours)
- \(b\) = bacterial population at time \(T_e\)
- \(B\) = bacterial population at time \(T_i\)

\(T_e\) and \(T_i\) are determined at appropriate intervals, selected according to the storage temperature of the samples.

\(pH\) Determinations.

All \(pH\) determinations were made with a Beckman H-2 \(pH\) meter equipped with glass electrodes.

\(\text{RESULTS}\)

The generation times of four strains of E. coli and three strains of A. aerogenes grown in reconstituted non-fat milk containing 9% serum solids and incubated at 50 and 90°F are shown in Table 1. The logarithmic growth phase usually occurred between the second and ninth days in milk incubated at 50°F and within the first 12 hr in milk incubated at 90°F. The data in this table also include the generation times computed during the interval between inoculation and 120 hr for each of the four trials. The logarithmic growth phase population was computed during the interval between inoculation and 120 hr for each of the four trials.

\(\text{TABLE 1. GENERATION TIMES OF COLIFORM ORGANISMS IN RECONSTITUTED NON-FAT MILK CONTAINING 9% SERUM SOLIDS}\)

<table>
<thead>
<tr>
<th>Incubation temperature</th>
<th>Generation time during:</th>
<th>Logarithmic growth phase</th>
<th>Interval from inoculation to maximum population</th>
<th>Logarithmic growth phase</th>
<th>Interval from inoculation to maximum population</th>
</tr>
</thead>
<tbody>
<tr>
<td>50°F</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
<td>(min)</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strain 1</td>
<td>516</td>
<td>942</td>
<td>34</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>strain 2</td>
<td>546</td>
<td>744</td>
<td>34</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>strain 3</td>
<td>522</td>
<td>774</td>
<td>33</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>strain 4</td>
<td>642</td>
<td>1038</td>
<td>39</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>A. aerogenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strain 1</td>
<td>540</td>
<td>870</td>
<td>33</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>strain 2</td>
<td>648</td>
<td>882</td>
<td>34</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>strain 3</td>
<td>636</td>
<td>1014</td>
<td>29</td>
<td>48</td>
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</tr>
</tbody>
</table>
and attainment of maximum population. The latter interval extended for 24 hr for all coliforms incubated in milk at 90 F, and for 13 to 21 days for samples held at 50 F.

The curves showing the trends in population of a representative strain of *E. coli* in reconstituted non-fat dry milk containing 9% serum solids and incubated at 32, 39, 50 and 90 F are shown in Figure 1. The population curves of this organism were representative of the seven strains of coliforms used in this study. During incubation at 90 F an initial coliform inoculum of 10 or less per ml reached a maximum number of approximately 10^9 cells per ml in one day. The population decreased only slightly through the third day and abruptly thereafter.

At 50 F initial numbers of 10 or less *E. coli* per ml attained a maximum population of 1.7 to 5.0 x 10^9 after 13 to 21 days. The *E. coli* strain represented by the data in Figure 1 attained a population of 2.7 x 10^9 per ml in 13 days. The *A. aerogenes* strains attained a maximum count of 1.5 to 2.6 x 10^9 per ml in 15 to 17 days. In general at 50 F the most rapid growth of the coliforms occurred between the second and ninth days, followed by a stationary phase and then a gradual decline in population.

An initial inoculum of 2500 *E. coli* cells per ml was added to reconstituted non-fat milk which was stored at 32 and 39 F (Figure 1). For the first 23 days of incubation the gradual decreases in population at the two temperatures were similar. In subsequent incubation the rate of decrease was greater at 39 than at 32 F. At both 32 and 39 F minor fluctuations occurred in the population curves of the seven coliform strains. The data in Figure 1 are typical of the results obtained.

The data in Figures 2 and 3 show the population trends of representative strains of *E. coli* and *A. aerogenes*, respectively, when inoculated into cottage cheese and incubated at 39, 50 and 55 F. The population of all strains of both organisms increased at 55 F and decreased at 39 F but at 50 F the numbers of *E. coli* increased while those of *A. aerogenes* decreased.

Non-inoculated control samples of milk and cheese incubated at the same temperatures and subjected to the same analyses as the inoculated samples consistently contained < 1 coliform per g.

The generation times of *E. coli* and *A. aerogenes* grown in cottage cheese at 50 and 55 F are shown in Table 2. The generation times of the *A. aerogenes* were slightly longer than those of *E. coli*.

A comparison of the generation times of *E. coli* grown in cottage cheese and reconstituted non-fat milk incubated at 50 F is shown in Table 3. These data demonstrate the superiority of the reconstituted nonfat milk over cottage cheese as a growth medium for coliforms. The most rapid growth of *E. coli* occurred within the first five days in the cottage cheese, but between the 5th and 10th days in the reconstituted nonfat milk.

![Figure 1. Population curves for Escherichia coli in reconstituted nonfat milk containing 9% serum solids and incubated at 32, 39, 50 and 90 F.](http://meridian.allenpress.com/jfp/article-pdf/27/7/197/2396614/0022-2747-27_7_197.pdf)

### Table 2. Generation Times of Coliform Organisms in Cottage Cheese

<table>
<thead>
<tr>
<th>Incubation temperature</th>
<th>55 F</th>
<th>50 F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interval in which generation time was obtained</strong></td>
<td><strong>E. coli</strong></td>
<td><strong>A. aerogenes</strong></td>
</tr>
<tr>
<td>(days)</td>
<td>(hr)</td>
<td>(hr)</td>
</tr>
<tr>
<td>0-5</td>
<td>31.4</td>
<td>32.8</td>
</tr>
<tr>
<td>0-10</td>
<td>35.2</td>
<td>38.0</td>
</tr>
<tr>
<td>0-15</td>
<td>38.7</td>
<td>43.3</td>
</tr>
<tr>
<td>0-20</td>
<td>42.5</td>
<td>47.3</td>
</tr>
</tbody>
</table>
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Table 3. Comparison of the Generation Times of One Strain of E. coli in Cottage Cheese and in Reconstituted Non-fat Milk Incubated at 50 F

<table>
<thead>
<tr>
<th>Interval for which generation time was obtained</th>
<th>Cottage cheese</th>
<th>Reconstituted nonfat milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>(generation time)</td>
<td>(hr)</td>
<td>(hr)</td>
</tr>
<tr>
<td>(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5</td>
<td>48.2</td>
<td>12.0</td>
</tr>
<tr>
<td>0-10</td>
<td>68.8</td>
<td>10.9</td>
</tr>
<tr>
<td>0-15</td>
<td>98.5</td>
<td>14.8</td>
</tr>
<tr>
<td>0-20</td>
<td>120.4</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Four strains of E. coli and three strains of A. aerogenes isolated from raw bulk tank milk and subsequently inoculated into sterile reconstituted nonfat milk showed decreases in population at 39 F and increases at 50 F. Kereluk et al. (11) obtained similar results with E. coli isolated from frozen meat pies.

The differences between results reported herein and those of other workers (2, 15, 16, 17) who reported growth of coliforms in raw and commercially pasteurized milk incubated at 37 to 39 F may be attributed to (a) differences in the strains of coliforms and (b) the symbiotic effect of mixed populations found naturally in non-sterilized milk. Perhaps a more extensive study of raw milk supplies would yield psychrophilic coliforms capable of growing in milk at 39 F or lower.

The greater decrease in numbers of coliform organisms at 39 than at 32 F may be attributed to the fact that permease enzymes are inactivated at both 32 and 39 F, but since some metabolic activity occurs at 39 F, toxic products may accumulate which are detrimental to cellular metabolism. Also, the fact that water has maximum density at 39 F may be a contributing factor to the greater death rate at this temperature.

At 50 F the generation time for strains of E. coli from time of inoculation to time of maximum count was similar to the time reported by Barber (3), but shorter than the time observed by Ingraham (9). However, at 90 F the generation times of E. coli during the logarithmic growth phase were comparable to those obtained in broth and reported by other workers (3, 9, 10). The generation time of A. aerogenes as reported by Jennison (10) was 8 to 12 min longer than found in this study. Age of inoculum and variation in medium used for propagation may account for this difference.

When incubated in cottage cheese E. coli tolerated lower temperatures than A. aerogenes. E. coli grew at 50 and 55 F; whereas A. aerogenes grew at 55 but not at 50 F. A decrease in cell numbers occurred at 39 F with both of the coliform organisms and the A. aerogenes population declined at 50 F. However, the diminution in cell population was negligible throughout the normal shelf-life of cottage cheese and coliforms persisted after the cheese had been stored for 15 to 20 days at 39 F. Harmon and Smith (8) reported that the shelf-life of cottage cheese stored at 42 F averaged 51% longer than corresponding samples held at 50 F.

In the reconstituted nonfat dry milk a coliform population of 1.7 to 5.0 x 10^6 per ml was reached within 13 to 21 days at 50 F, however, in the cottage cheese the maximum count of approximately 10^9 per g occurred between the 20th and 35th days in samples stored at 55 F. The differences in maximum popu-
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lation between the two products were attributed to the lower pH and the presence of competitive organisms in cottage cheese. The coliforms are sensitive to pH below 5.0. Harmon and Smith (8) found that the coliform population declined in cottage cheese samples held at 42 F which had an initial and terminal pH of 4.8 or less. When the initial and terminal pH was above 5.1 continuous increases in numbers occurred.

Several pH determinations were made on cottage cheese samples during incubation. In one trial the initial pH was 5.1 and no change in pH occurred during the first 14 days at 39, 50 or 55 F. This may explain the rapid growth and shorter generation times during the first 10 days of growth. In another trial the initial pH of the cheese was 5.4 and at 60 days the pH readings were 5.3, 4.8 and 4.4 at 39, 50 and 55 F, respectively. This indicates that the decline in cell numbers at 39 F was primarily due to the effect of temperature rather than pH.

REFERENCES

3. Barber, M. A. Rate of multiplication of Bacillus coli at different temperatures. J. Infect. Dis. 5:379. 1908.