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ABSTRACT

Preliminary incubation of raw milk samples to evaluate bacterial quality has been a commonly recommended practice. The presumption has been that selective growth of a segment of the microflora associated with unsanitary methods and conditions would be enhanced. The present work involved a theoretical approach to evaluate this presumption. Pure cultures of *Pseudomonas aeruginosa, P. fluorescens*, and *Escherichia coli* as well as enriched mixed cultures of psychrotrophs and mesophiles were used. The generation time of each culture was determined at various temperatures. An analysis of covariance showed the coefficients of generation time related to temperature were not significantly different. Thus, preliminary incubation in the range of the temperature now recommended does not have a selective effect for specific groups of microorganisms.

INTRODUCTION

Many methods have been suggested to improve the parameters for determining bacterial counts on raw milk as a means for evaluating its quality. One of the most common methods involves preliminary incubation for 18 h at 12.8°C (55°F) prior to performing a plate count (2). This technique was thought to enhance growth of a segment of the microflora commonly associated with unsanitary methods and conditions at the farm (3).

The relation between preliminary incubation and sanitary conditions on the farm more recently has been shown to be rather imprecise (1, 4). Furthermore, the relation between various methods of evaluating quality is likewise rather imprecise (7, 8). The lack of precision may be related to the high variability in bacterial quality from one sampling period to another (5).

This work was designed to determine if preliminary incubation enhanced the growth of one segment of common microbial contaminants more than another. The goal was to obtain a more nearly correct theoretical statement on the usefulness of preliminary incubation.

MATERIALS AND METHODS

Cultures

Pure cultures of *Escherichia coli, Pseudomonas aeruginosa*, and *P. fluorescens* were obtained from the University of Nebraska-Lincoln, Department of Food Science and Technology culture collection. *Escherichia coli* was chosen to represent a typical mesophile, and *P. aeruginosa* and *P. fluorescens* were chosen to represent the broad spectrum of contaminants with pseudomonads. They were propagated in m-Plate Count Broth (PCB; Difco Laboratories, Detroit, MI) with incubation at 32°C and storage at 5°C. To provide more rapid growth than in heat-sterilized reconstituted nonfat milk solids, PCB was chosen.

A mixed flora of naturally occurring psychrotrophs was obtained by enrichment. Samples of 100 ml of mixed raw Grade A milk from bulk tank trucks were incubated for 1 wk at .5°C after which 5 ml was transferred to 100 ml of another sample of fresh milk from bulk tank trucks and incubated at .5°C for a week.

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TABLE 1. Effects of temperature on rate of growth of microorganisms commonly found in raw milk.

Organism temperature	Generation time (h)	Log generation time
Escherichia coli		
10	14.81	1.171
15	2.45	.389
20	1.42	.152
25	.74	−.134
30	.60	−.222
Pseudomonas aeruginosa		
10	21.11	1.324
15	4.33	.636
20	2.04	.310
25	1.18	.072
30	1.15	.061
Pseudomonas fluorescens		
10	8.49	.929
15	1.61	.207
20	1.41	.149
25	.99	−.004
30	.73	−.137
Mesophiles		
10	7.96	.901
13	3.97	.599
20	1.34	.127
25	.77	−.114
32	.45	−.347
Psychrotrophs		
.5	14.65	1.166
2.5	9.95	.998
9	3.84	.584
15	1.71	.233
20	1.16	.064
25	.84	−.076

This enrichment process was repeated six times. The resulting culture was then propagated in PCB with growth at 25°C and storage at 5°C.

To obtain a mixed flora of mesophiles, an enrichment process was used. Raw Grade A milk was incubated at 32°C until a standard plate count of approximately 5×10^7 cfu/ml was obtained. A 1% inoculum was then transferred into fresh raw Grade A milk and incubated at 32°C until the count was approximately 5×10^7 cfu/ml. This process was repeated twice, and the resulting culture was propagated in sterile 9% reconstituted nonfat milk solids with incubation at 32°C and storage at 5°C.

Generation Times

To determine generation times, an inoculum of 10^3 to 10^4 actively growing cells was used. The pure cultures and the psychrotrophs were grown in PCB, and the mesophiles were grown in sterile 9% reconstituted nonfat milk solids. Plate counts were determined according to *Standard Methods for the Examination of Dairy Products* (6). All generation times were calculated using data obtained from the log of the growth cycle. The specific generation time was determined by the slope of the growth curve as established by the method of least squares. For each temperature two independent generation times were determined and averaged for each culture.

RESULTS AND DISCUSSION

Table 1 shows the relation between incubation temperatures, generation times, and log generation time of the pure cultures and the enriched mixed populations. As the temperature increased the generation time decreased. There was an inverse, linear relation between temperature and the log of the respective generation time. The correlation was −.90 or better for the pure cultures and −.98 or better for the enriched mixed populations.

The data in Table 1 were subjected to analysis of covariance with the temperature as the covariate, organism as classification, and log generation time as the response. The correlation coefficient for this model was −.95. The slope coefficients of generation time related to tem-

TABLE 2. The relation between temperature and the log of the generation time.

Organism	Slope coefficient
Escherichia coli	−.066
Pseudomonas aeruginosa	−.062
Pseudomonas fluorescens	−.047
Mesophiles	−.056
Psychrotrophs	−.052

perature for each of the groups of organisms range from −.047 to −.066 (Table 2). These were not significantly different ($P>.1$). Thus, changes of temperature had an equal effect on each culture.

Because the slope coefficients were not significantly different, the results therefore indicated that the temperature used for preliminary incubation does not have a selective effect for specific groups of microorganisms. However, a temperature of 5°C or lower would be expected to have a selective effect. These results are in harmony with common observations that an acceptable correlation is observed between plate count determinations with and without preliminary incubation of raw samples. However, high correlation is generally associated with a few very high counts (5). The relatively low counts have little effect on correlation analysis but are critical for modern quality assurance.

Although preliminary incubation is generally thought to increase selectively the numbers of microorganisms that indicate unsanitary conditions on a farm, our results show that the impact is similar on all groups of microorganisms and does not cause selection of any one group. Therefore, we conclude that the preliminary incubation temperature is not critical and does not add reliability to estimates of bacterial quality of raw milk.

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