

INFLUENCE OF 32 AND 37 C INCUBATION TEMPERATURES ON COUNTS OF COLIFORM BACTERIA OF MILK¹

D. L. MALLET, R. T. MARSHALL AND J. E. EDMONDSON

Department of Food Science and Nutrition
University of Missouri, Columbia 65201

(Received for publication January 9, 1969)

ABSTRACT

Samples of raw and pasteurized milk were plated in desoxycholate lactose agar and incubated at 32 C \pm 1.0 and 37 C \pm 1.0 for enumeration of coliform bacteria. The lower temperature produced counts comparable to those at 37 C. Pasteurized samples yielded coliform-type bacteria which had 5 different IMViC test patterns, whereas 6 patterns were found among the isolates from raw milk. The most predominant pattern was - +++ and existed among 33% of the isolates.

This study was designed to determine if incubation temperatures of 32 or 37 C were selective toward any particular type or types of coliform organisms (as characterized by the IMViC test) and whether more organisms could be recovered from raw or pasteurized milk using either incubation temperature. *Standard Methods* (1) now requires the use of 32 C.

The literature is somewhat confusing in respect to the optimum incubation temperature for coliform bacteria. Boniece and Mallman (3) reported the optimum growth temperature for *Escherichia coli* and *Aerobacter aerogenes* as in the range of 37 to 39 C and the most favorable incubation temperature for the entire coliform group as 35 C. Murry (8) found that more colonies grew on MacConkey's agar at 30 than at 37 C when raw and pasteurized milks were examined. However, after examining 416 samples of milk, Lawton (7) found no significant difference in the number of coliform colonies yielded at 32 and 35 C. Thomas, et al., (9) isolated bacteria of the coli-aerogenes group from 825 samples at 30 C and from 735 at 37 C. They concluded that 30 C is more selective for *Aerobacter* and *Klebsiella* species whereas 37 C is more selective for *E. coli*.

Geldreich, et al. (5) indicate that there are 12 common coliform IMViC types of which 1 and 2 are *E. coli* and are of fecal origin. These are designated + + - - and - + - -, respectively, in the IMViC series. The Eijkman test for production of acid and gas in MacConkey's broth at 44 C is used for detecting *E. coli* type 1 which reacts + + - - to the IMViC series (2, 6).

Results of previous research attempts may have been influenced by the type of sample selected. Some workers examined water (3, 9) whereas others used milk (7, 8).

MATERIALS AND METHODS

Samples

Samples of Grade A raw milk were collected at the pasteurization plant or the producing farm. Samples originated from the University of Missouri Holstein-Jersey and Foremost Guernsey herds, bulk deliveries from Kansas City, Missouri and from selected farms in the Columbia, Missouri milkshed.

Pasteurized milk samples were taken from University cafeteria dispensers and from retail stores. Samples were held at 4 C and plated within 8 hr of collection.

Examination of raw and pasteurized samples

Platings, in accordance with *Standard Methods for the Examination of Dairy Products* (1), were made at 1:1 and 1:10 dilutions in duplicate using desoxycholate lactose medium. One set of plates was placed at each test temperature.

After counting, typical colonies were picked from plates incubated at both temperatures for 17 samples of pasteurized milk and 26 samples of raw milk. Colonies were placed in lactose broth and incubated at the temperature at which they grew. Tubes were examined for gas production after 24 hr. Cultures in tubes showing no gas were discarded. Cultures of Gram-negative bacilli from tubes showing gas production were subjected to the series of IMViC tests (2).

Study of six isolated strains

Two sets of lactose broth tubes were inoculated with each of six strains which represented the different IMViC reaction patterns observed. One set was incubated at 32 C and the other at 37 C for 24 hr. These were then plated in desoxycholate lactose agar at dilutions to produce 15 to 150 colonies per plate and incubated for 24 hr at the temperature of previous incubation.

To eliminate the possibility that temperature of culturing in the broth might have influenced the counts in the previous experiment, each of the six strains was inoculated into a single tube of lactose broth and incubated 24 hr at 37 C before plating and incubating at the two test temperatures.

Counts from each of these experiments were converted to logarithms, and tests were performed to determine whether there were statistically significant differences between the means (4).

RESULTS AND DISCUSSION

Pasteurized milk

Coliform count comparisons were made on 190 samples of pasteurized milk. A distribution of counts

¹Contribution from the University of Missouri Experiment Station. Journal Series Number 5562.

is shown in Table 1. No colonies grew at either temperature on plates made from 48 samples. Colonies were more numerous for 62 samples incubated at 32 C and for 52 samples incubated at 37 C. Counts were equal for 28 samples. Logarithmic mean counts differed by only .0014 in favor of the 32 C temperature. Samples which produced no growth were not included in the average. These data indicate that the lower temperature is at least as satisfactory as the higher from the standpoint of numbers of coliforms detected.

TABLE 1. DISTRIBUTION OF COLIFORM COUNTS OF PASTEURIZED MILK SAMPLES INCUBATED AT 32 C AND 37 C

Coliforms/ml	Number of samples within class	
	at 32 C	at 37 C
Less than 1	68	67
1 to 10	97	101
11 to 100	22	19
More than 100	3	3
	190	190

Twelve coliform types were cited by Geldreich, et al. (5), and five of these types were isolated from the pasteurized samples (Table 2). The IMViC reactions were + + - -, - + - -, - - + +, - + + +, and - + - +. These were found among 34 isolates equally divided between the two temperatures, one colony having been picked from each of 17 pairs of plates. Three cultures produced no gas, two from 32 C and one from 37 C plates.

TABLE 2. COLIFORM/ML OF PASTEURIZED MILK AND TYPES ISOLATED FROM PLATES INCUBATED AT 32 C AND 37 C.

Sample	IMViC Reaction		IMViC Reaction	
	Coliforms/ml (32 C)		Coliforms/ml (37 C)	
1	3	- + + +	3	- + - +
2	11	- + + +	9	- + - +
3	21	- + + +	15	+ + - -
4	5	- + + +	9	+ + - -
5	23	- + + +	20	- + + +
6	136	- + + +	104	- + + +
7	9	- + + +	9	- + + +
8	20	- + + +	14	- + - +
9	30	- + + +	26	- - + +
10	11	+ + - -	14	+ + - -
11	7	+ + - -	1	+ + - -
12	3	+ + - -	3	+ + - -
13	13	- - + +	19	- + - +
14	3	- - + +	2	+ - - -
15	2	- + - -	1	- + - -
16	1	*	2	- - + +
17	2	*	2	*

*No gas produced in lactose broth; therefore, no IMViC test made.

TABLE 3. DISTRIBUTION AND MEANS OF COLIFORM COUNTS OF RAW MILK SAMPLES INCUBATED AT 32 C AND 37 C.

Coliforms/ml	Number of Samples	Mean Counts at 32 C	Mean Counts at 37 C
Less than 10	26	*	*
10 to 100	59	30	32
110 to 1000	21	434	439
More than 1000	7	4380	3400

*Not applicable

Seven pairs of plates produced organisms with the same IMViC reactions. However, nine were not the same. The type predominating reacted - + + + to the test series. There were 11 of these cultured. Three *E. coli* cultures were picked from 32 C plates and five from 37 C plates.

Raw milk

Coliform count comparisons were made on 113 samples of raw milk. No colonies grew at either temperature on plates of 1:10 dilutions of 26 samples. The 32 C temperature produced 37 higher counts, whereas 30 were higher when plates were incubated at 37 C. Counts were equal for 46 samples. The arithmetic mean counts shown in Table 3 were essentially the same for the two temperatures with the exception of the "more than 1,000" classification. However, these means were greatly influenced by two extremely high counts at 32 C. These data verify those from the pasteurized milk experiment with respect to the positive suitability of 32 C incubation.

It is interesting that 75% of the raw samples produced coliform counts of 100 or less per milliliter.

Six of the 12 coliform types, previously mentioned, were isolated from 52 colonies picked from duplicate plates of raw milk held at the two temperatures (Table 4). Five of these were the same types as isolated from the pasteurized samples. The additional type reacted + + - + to the IMViC test. Thirty-two isolates gave the IMViC reaction of - + + +. The same strain predominated in the pasteurized samples.

Twice as many isolates from raw milk grown at 32 C failed to produce gas as those grown at 37 C, 6 vs. 3. However, the numbers are probably too small to imply significance. Logarithmic mean counts differed only by 0.1048 in favor of the lower temperature.

Study of six isolated strains

When the strains representing the 6 IMViC test patterns were grown out at 32 C and 37 C in lactose broth, five of the six produced higher counts at the lower temperature. However, the average arithmetic mean difference was only 7 colonies per plate at dilutions which produced 15 to 150 colonies per

TABLE 4. COLIFORMS/ML OF RAW MILK AND TYPES ISOLATED FROM PLATES INCUBATED AT 32 C AND 37 C.

Sample	32 C			37 C		
	Coliforms/ml	IMViC Reaction	Coliforms/ml	IMViC Reaction	Coliforms/ml	IMViC Reaction
1	70	- + + +	70	+ + - -		
2	70	- + + +	60	- + + +		
3	90	- + + +	40	- + + +		
4	40	- + + +	30	- + + +		
5	670	- + + +	860	- + + +		
6	66	- + + +	50	- + + +		
7	70	- + + +	70	- + + +		
8	2560	- + + +	2624	*		
9	500	- + + +	500	- + + +		
10	126	- + + +	32	- + + +		
11	38	- + + +	40	- + + +		
12	3	- + + +	3	- + + +		
13	50	- + + +	52	+ + - -		
14	11	- + + +	10	+ + - -		
15	5688	- + + +	5000	- + + +		
16	6312	- + + +	7232	- + + +		
17	49	- - + +	45	- + + +		
18	20	- + - +	10	- - + +		
19	10	+ + - -	0	**		
20	1	+ + - +	0	**		
21	9	*	9	- + + +		
22	10	*	10	- + + +		
23	364	*	532	- + + +		
24	2	*	1	- + + -		
25	2015	*	2275	*		
26	6500	*	2340	*		

*No gas produced in lactose broth; therefore, no IMViC test made.

**No test possible.

plate, and logarithmic expression of the counts produced an average difference of only 0.088. A "t" test for differences between the logarithmic means failed to show a significant difference between the counts obtained by the two methods.

When the same six strains were grown out in lactose broth at 37 C, plated in duplicate and incubat-

ed at both 32 C and 37 C, four of the six strains produced more colonies at 32 C. Again, the mean differences were small (arithmetic mean difference = 7; logarithmic mean difference = 0.082), and the "t" test for difference between the logarithmic means failed to show significance.

These results indicate that incubation at 32 C produces counts of coliform bacteria from milk which are at least as high as those produced when 37 C is used. Only 6 of 12 common types (as determined by IMViC reaction) were tested, but both *E. coli* (14% of isolates) and *A. aerogenes* (7% of isolates) were included. The isolate most frequently encountered (IMViC reaction - + + +) was present in at least 33% of the samples.

REFERENCES

1. American Public Health Association. 1967. Standard Methods for the Examination of Dairy Products. 12th Ed. American Public Health Assoc., New York.
2. American Public Health Association. 1955. Standard Methods for the Examination of Water, Sewage and Industrial Wastes. 10th Ed., American Public Health Assoc., New York. pp. 391-393.
3. Boniece, J. R., and W. L. Mallman. 1950. The optimum incubation temperature for the primary isolation of coliform organisms. J. Am. Water Works Ass. 42:155.
4. Freund, J. E. 1952. Modern Elementary Statistics. Prentice-Hall, Inc. Englewood Cliffs, N. J.
5. Geldreich, E. E., H. F. Clark, P. W. Kabler, C. B. Huff, and R. H. Borner. 1958. The coliform group II reactions in EC media at 45 C. Appl. Microbiol. 5:341.
6. Harrigan, W. F., and M. E. McCance. 1966. Laboratory Methods in Microbiology. Academic Press, London., pp. 94-95.
7. Lawton, W. C. 1955. A comparison of 32 C and 35 C as incubation temperatures for the coliform count of milk and cream. J. Milk Food Technol. 18:280.
8. Murry, J. G. 1953. A comparison of 30 C and 37 C as incubation temperatures in the presumptive coli-aerogenes test for raw and pasteurized milk. Soc. Appl. Bacteriol. 16:24.
9. Thomas, S. B., P. M. Hobson, and R. G. Druce. 1959. Coli-aerogenes bacteria in farm water supplies. J. Appl. Bacteriol. 22:32.