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RAPID DETECTION OF COLIFORMS IN PASTEURIZED MILK*

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INTRODUCTION

There are numerous tests available for detection of coliforms in pasteurized milk. Primarily completion of these tests require a minimum of 18-72 hr (ISI, 1962). Such a delay impose limitation of the tests to be used, as pasteurized milk is likely to be consumed before the tests are completed. Attempts have been made to develop rapid tests for the detection of coliforms by different workers (Forg, 1956; Futschik, 1956; Levine *et al.*, 1956; Adamczyk and Chmielowski, 1975; Newman and O'Brien, 1975; Moran and Witter, 1976; Sudarsanam and Nambudripad, 1978). Most of these tests have not been employed for pasteurized milk. The present investigation deals with development of a simple test which may detect coliforms in pasteurized milk in about 6 hr — a period before pasteurized milk would be sent for distribution.

MATERIALS AND METHODS

One hundred and four samples of freshly pasteurized milk were collected from the Experimental Dairy of the Institute. Equal volumes of pasteurized milk were mixed with different broth media of double strength (Table 2). One ml aliquot was transferred to a test tube (100 mm × 6 mm). A layer of soft agar (0.5% w/v) was gently poured into the test tube so as to form a distinct layer over the pasteurized milk plus broth. After plugging, the test tube was transferred to an incubator at 37°C and examined every half an hour for gas production at the junction below the column of the soft agar, with the help of a magnifying lens. Initial trials indicated that the pasteurized milk had a few coliforms and hence these organisms were concentrated by centrifuging 100 ml of pasteurized milk at 4,000 rpm for 20 min. One ml of this centrifuged milk was mixed with 1 ml of the best selected broth medium (double strength) and examined for gas production as indicated above. The detailed procedure has been illustrated in Fig. 1. The effect of different volumes of the best selected broth medium and incubation temperature on the gas production was also studied. Coliform counts in all the pasteurized milk samples were estimated as given by ISI (1962), using E.M.B. agar.

RESULTS AND DISCUSSION

Incidence of coliforms in pasteurized milk: Out of a total of 104 samples tested, coliforms present in pasteurized milk ranged between 0 and 4,500/ml (data not presented). Such a wide variation in coliform counts of pasteurized milk is not unexpected in view of the differences in the initial quality of milk before pasteurization, post-pasteurization contamination, heat resistant characteristics of some strains and reactivation of heat injured cells after pasteurization (Provan and Rowlands, 1939; Crossley, 1946; Fustschik and Sachslehner, 1956; Singh, 1970).

For convenience, the average coliform counts have been arbitrarily categorized into 4 ranges namely <1.0, 1-10, 11-100 and >100/ml. The per cent distribution of coliforms falling in

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these ranges was 8.6, 38.5, 37.5 and 15.4, respectively (Table 1).

TABLE 1

Distribution of coliforms in pasteurized milk

Coliform counts/ml (Range)	No. of samples	Per cent distribution
< 1	9	8.6
1 — 10	40	38.5
11 — 100	39	37.5
> 100	16	15.4
Total	104	

Effect of incorporation of different broth media: The effect of incorporation of 9 different broth media in pasteurized milk on the time taken to detect gas production by coliforms is shown in Table 2. Of all the broth media used, MacConkey's broth was considered best because of its being a selective medium for the coliforms as well as it showed change in colour due to the presence of acid-base indicator in addition to the gas production. In general, the time taken with this broth medium for gas production was comparatively less. Attempt to incorporate nutrients in broth media which may help in the repair of heat injured cells did not reduce the time of gas production (Table 2).

Fig. **Procedure for detection of gas produced by coliforms.**

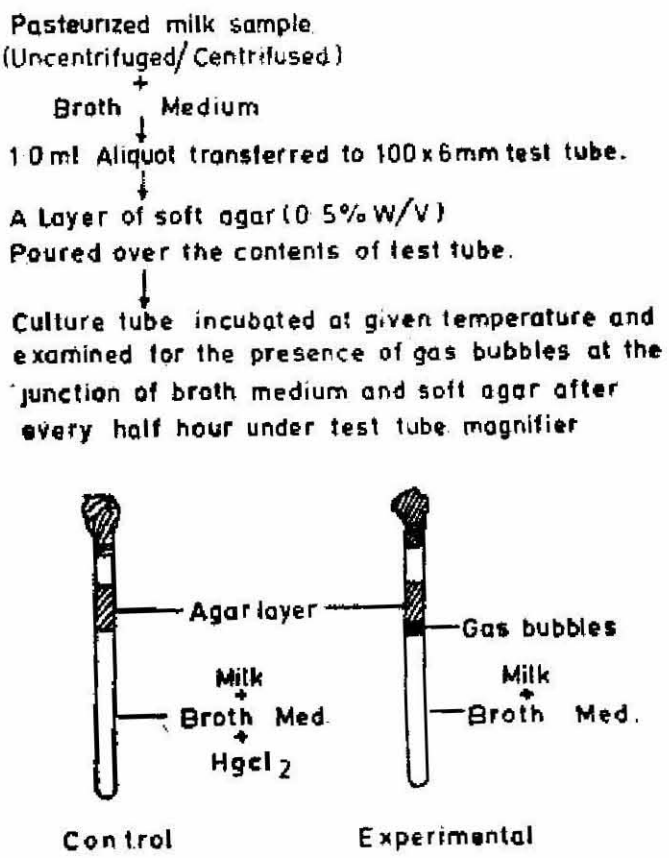

TABLE 2

Effect of incorporation of different broth media in pasteurised milk on gas production by coliforms

Counts*	Peptone	Peptone bile	Yeast ext. bile	Beef ext. bile	Tryptose yeast extract beef ext. bile	E.C.	L.S.	Mac-Conkey	ZH-45
				(Time in hr for gas production at 37°C)					
<1	—	—	—	—	—	—	—	—	—
1—10	10.4	10.5	9.8	10.0	10.1	9.7	9.5	9.5	9.8
11—100	7.4	7.4	7.1	7.4	7.4	7.2	7.2	7.2	7.2
>100	—	—	—	—	—	—	—	—	—

*Counts per ml (Range) L.S.—Lauryl sulphate

Effect of centrifugation on coliform counts: From Table 2 it is seen that time taken for gas production was as long as 10.5 hr; when the coliform counts in pasteurized milk were 1-10/ml. it was only 7.4 hr. Therefore, for early detection of gas production by the coliforms, it was considered to concentrate the coliforms in pasteurized milk by centrifugation. The effect of centrifugation on coliform counts with bottom layer and supernatant is shown in Table 3. It is observed that centrifugation techniques facilitated concentration of coliforms in bottom layer. Coliform counts increased by 8 to 10-folds, when the counts in uncentrifuged milk were <100/ml. Coliform counts above 100/ml showed only 2.5-folds increase after centrifugation. Further, when the bottom layer of centrifuged milk was mixed with MacConkey's broth medium the time taken to produce the gas was significantly reduced as compared to uncentrifuged milk (Table 4). Hence subsequent trials were carried out using centrifuged milk only.

TABLE 3

Effect of centrifugation on coliform counts in pasteurized milk

Coliform counts/ml (Range)	Average coliform counts per/ml		
	Uncentrifuged milk	Centrifuged milk 1 ml of bottom layer	Centrifuged milk Supernatant
< 1	0.3	3.5	0.0
1 — 10	5.6	44.9	0.7
11 — 100	37.4	274.5	3.5
>100	313.3	793.3	23.0

Effect of volume of broth medium: The effect of 3 different volumes (0.5, 1.0 and 2.0 ml) of the best selected broth medium (MacConkey's broth) was studied. The results revealed (Table 5) that incorporation of 0.5 and 1.0 ml MacConkey's broth into 1.0 ml each of centrifuged milk significantly reduced the time required for gas production as compared to 2.0 ml of broth medium. Delayed period of gas production in case of 2.0 ml of broth (Table 5) might have been due to dilution effect of larger volume used.

TABLE 4

Effect of centrifugation of pasteurized milk on gas production by coliforms

Coliform counts/ml (Range)	Pasteurized milk + MacConkey's broth	
	Uncentrifuged	Centrifuged*
	(Time in hr for gas production at 37°C)	
< 1	18	18
1 — 10	10.4	7.5
11 — 100	9.1	6.8
> 100	6.9	5.4

*Only pasteurized milk was centrifuged.

TABLE 5

Effect of different volumes of Mac Conkey's broth medium on gas production by coliforms in pasteurized milk after centrifugation

Coliform counts/ml (Range)	1 ml centrifuged milk + Different vols. of MacConkey's broth		
	0.5 ml	1.0 ml	2.0 ml
	(Time in hr for gas production at 37°C)		
< 1	12	12	12
1 — 10	7.3	7.5	8.9
11 — 100	6.5	6.6	7.2
> 100	5.0	5.0	5.1
Average time	6.8	6.8	7.4

Effect of incubation temperature: A comparison of temperature of incubation at 37°C and 41.5°C on the time of gas production is shown in Table 6. For the different ranges of coliforms in pasteurized milk, time taken to detect gas production at 37°C varied between 5.0 and 12.0 hr, while at 41.5°C it was significantly less (4.2-8.5 hr). The recent trend emphasizes detection of faecal coliforms (Geldreich, 1966). Generally incubation temperature of 44.5°C has been recommended for the detection of such coliforms (Mundt and Rai, 1963; Geldreich, 1966; Geetha and Nambudripad, 1968). On the other hand, wide range of temperatures (41°-46°C) has been recommended (DeFigueiredo and Jay, 1976). Recently Francis *et al.* (1974) have also used 41.5°C as incubation temperature for the detection of faecal coliforms. Therefore, the present test may be applied for the detection of faecal coliforms also.

TABLE 6

Effect of incubation temperature (37 and 41.5°C) on gas production by coliforms in pasteurized milk

Coliform counts per ml (Range)	Incubation temperature	
	37°	41.5°C
	(Time in hr for gas production)	
< 1	12	8.5
1 — 10	7.5	6.4
11 — 100	6.7	5.7
> 100	5.0	4.2

This test was faster in detecting the gas production by coliforms as compared to the rapid test reported earlier by Sudarsanam and Nambudripad (1978). The difference in the time of gas production may be due to employment of centrifugation step in the present investigation for concentrating in the organisms in pasteurized milk. This as also possible due to less interaction effect between coliform and non-coliforms, since the present method has recommended incorporation of MacConkey broth for detection. This broth medium is known to inhibit non-coliforms (MacConkey, 1901). However, efficacy of the test in presence of enterococci and anaerobic spore formers needs to be further investigated. This test can be conveniently used for the line testing programme in a dairy plant.

SUMMARY

The present study was undertaken to develop a simple test for rapid detection of coliforms in pasteurized milk.

Centrifugation of pasteurized milk at 4,000 rpm/20 min increased the coliform count in the bottom layer by 8 to 10-folds. Use of this centrifuged milk considerably reduced the time for gas production due to coliforms by 1.5 to 3.0 hr as compared to uncentrifuged milk. Out of the three volumes (0.5, 1.0 and 2.0 ml) of MacConkey's broth, 1.0 ml volume indicated minimum time of gas production. Incubation at 41.5°C was superior to 37°C. Statistical analysis of the data has revealed significant difference in the time of gas production due to centrifugation, volume of broth medium and incubation temperature. The recommended procedure for the rapid detection of gas production by coliform involved centrifugation of 100 ml pasteurized milk and mixing 1.0 ml of the centrifuged milk (bottom layer) with 1.0 ml of MacConkey's broth (double strength). One ml of the mixed sample was incubated at 41.5°C and the gas produced was detected under a layer of soft agar. This test could detect coliforms within 6.4 hr including the period of centrifugation when pasteurized milk contained coliforms upto 10/ml. Pasteurized milk samples having coliform counts of 11-100 and >100/ml showed positive results within 5.7 hr and 4.2 hr, respectively. The application of this test in quality control programme has been discussed.

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