

Repair and Enumeration of Injured Coliforms by a Plating Procedure

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Received for publication 9 December 1974

Surface plating of coliforms on Trypticase soy agar, followed by 1 to 2 h of incubation at 25 C and subsequent overlay with violet red bile agar, was found to be a useful method for the repair and enumeration of coliforms injured by freezing.

Injured coliforms in frozen foods may not be enumerated by selective media unless they are first allowed to repair. Substantial repair can occur in a nonselective medium, such as Trypticase soy broth (TSB), within 1 h at 25 C (3, 4). The disadvantage of this method is that coliforms vary in the time required for repair and, because of this, multiplication of certain cells may occur before other cells can repair. In an effort to avoid this limitation in the enumeration of injured as well as uninjured cells, we studied repair on Trypticase soy agar (TSA) followed by overlay of violet red bile agar (VRBA) to suppress colony formation by non-coliforms. Various adaptations of this method are now being studied, and this note reports some of our preliminary findings.

Five coliform isolates from frozen foods and stock culture *Escherichia coli* K-12 were tested for their recovery by different methods. Each culture was grown on TSB for 4 to 5 h at 37 C in a shaker incubator, centrifuged, washed, and suspended in sterile meat broth to give 2×10^8 cells/ml; this suspension was frozen at -20 C for 18 to 24 h (3). After thawing at 10 C, serial dilutions were made in 0.1% peptone water. Cells surviving at different levels of viability were measured by plating appropriate dilutions of the samples in duplicate as follows. (i) To measure the number of total survivors, 0.5 ml of the sample was surface plated on TSA; incubation was at 35 C for 24 h. (ii) To measure the number of uninjured survivors, 0.5 ml of the sample was surface plated on VRBA, incubated 1 h, overlaid with 10 to 12 ml of tempered (45 C) VRBA and incubated 24 h at 35 C. Typical red-to-pink colonies were enumerated for procedures (ii), (iii), and (iv). (iii) To measure the number of injured cells after repair by using agar medium, 0.5 ml of the sample was surface

plated on 12 ml of TSA, incubated 2 h at 35 C, overlaid with 10 to 12 ml of VRBA and incubated 24 h at 35 C. (iv) To measure the number of injured cells after repair in liquid medium, 1 ml of thawed sample was incubated in 9 ml of TSB for 1 h at 25 C; 0.5 ml of the sample was surface plated on VRBA with an overlay of VRBA. Details of the surface plating procedure have been described earlier (2).

Colonies that developed on the TSA plates were considered to represent 100% of the cells that had survived freezing; these would be from injured as well as uninjured cells (Table 1). Repair of injured cells in TSB was used as a reference since this method has been studied extensively (3, 4). The high count obtained for isolate 3 (133%) and possibly 1 (111%) was interpreted as having resulted from early multiplication of certain noninjured cells during the

TABLE 1. Recovery of coliforms from frozen meat broth after repair in agar or liquid medium

Isolates	Enumeration ^a (%)		
	Uninjured survivors ^b	After repair in: ^c	
		TSA	TSB
1	37	108	111
2	60	79	102
3	38	109	133
4	33	73	47
5	13	97	82
<i>E. coli</i> K-12	1	94	47

^a Total survivors of the freezing as enumerated on TSA by surface plating were considered as 100%.

^b Difference between total survivors and uninjured survivors represents injured cells.

^c Any increase in recovery over uninjured survivors was due to repair.

TABLE 2. Recovery of coliforms from 18 commercial ice cream samples by different plating methods

Determinations	Plating method		
	Pour plating (VRBA)	VRBA overlay after 1 h on: ^a	
		VRBA	TSA
Total colonies ^b	51	199	264
Logarithmic avg/ml	2.6	8.8	10.3
Range/ml	0 to 10	0 to 29	1 to 45
With ≥ 10 /ml (%)	6	44	67
BGLB ^c positive (%)	80	92	84

^a For explanation see the text.

^b Total coliform colonies from the 18 samples. One milliliter of each sample was plated in duplicate plates.

^c BGLB, brilliant green-lactose bile broth.

repair treatment in TSB. This could be a definite limitation to the use of this repair treatment, especially in regulatory surveillance.

Repair on TSA followed by use of the VRBA overlay generally allowed more complete repair of the injured population without imposing problems caused by cell multiplication. Conceivably a somewhat longer time or more appropriate medium used for repair would allow more of the injured cells to be repaired and enumerated.

In addition to pure cultures, 18 samples of commercial ice cream were studied. Immediately after thawing, 0.5-ml aliquots were plated in duplicate by the conventional pour-plating method with VRBA (1) and by surface plating on VRBA and on TSA. After being at 25 C for 1 h, the surface-inoculated plates were then overlaid with 10 to 12 ml of VRBA. All plates were incubated 24 h at 35 C, after which all pink-to-red colonies were counted. A proportionate number (approximately 25%) of representative colonies from each plating method was picked into brilliant green-lactose bile broth. Gas production after 48 h at 35 C was used as presump-

tive evidence that the colonies were coliforms. The data are summarized in Table 2.

The conventional pour-plating procedure enumerated fewer coliforms than either of the two surface-plating methods, which confirmed a previous report (2) on the inability of this method to enumerate coliforms adequately. Repair on TSA, followed by the overlay of VRBA, gave the highest coliform counts. The data confirms an earlier study (2) which showed that surface plating on VRBA avoids lethal effects of melted (45 C) VRBA on injured cells; furthermore, repair of some injured cells can occur when surface plated because of the time required for equilibration of the sample with selective ingredients in the VRBA (unpublished data).

From these and other data, we feel that the inoculation of a solid nonselective medium followed by the addition of a selective medium after a period of repair offers a useful method for the enumeration of injured organisms. Currently, our investigations deal with optimizing and possible further simplification of the repair and enumeration procedure. These involve plating procedures, time and temperature for repair, and the repair medium.

This investigation was supported by a contract grant no. 223-74-2098 from the Food and Drug Administration.

We thank the Dairy Division of the North Carolina Department of Agriculture for the ice cream samples used in this study. The technical assistance of Carol Howell is gratefully acknowledged.

LITERATURE CITED

1. American Public Health Association, Inc. 1972. Standard methods for the examination of dairy products, 13th ed., p. 157. American Public Health Association, Inc., New York.
2. Ray, B., and M. L. Speck. 1973. Enumeration of *Escherichia coli* in frozen samples after recovery from injury. *Appl. Microbiol.* **25**:409-503.
3. Ray, B., and M. L. Speck. 1973. Discrepancies in the enumeration of *Escherichia coli*. *Appl. Microbiol.* **25**:494-498.
4. Warseck, M., B. Ray, and M. L. Speck. 1973. Repair and enumeration of injured coliforms in frozen foods. *Appl. Microbiol.* **26**:919-924.