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Plating Procedure for the Enumeration of Coliforms from Dairy Products†

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A "repair-detection" procedure consisting of pour plating of food samples with Trypticase soy agar, followed by 1-h repair incubation at room temperature and subsequent overlay with violet red bile agar, was found to be an effective method for the detection of injured and uninjured coliforms from dairy products. This method was relatively less effective for the detection of coliforms in many semipreserved foods as compared with dairy products, but more effective than the most-probable-number method.

We previously reported a surface-overlay plating technique that could be used effectively in the detection of injured coliforms from food samples (6). Since then, we have studied various modifications of this method, such as plating methods (pour and surface), plating medium (plate count agar and Trypticase soy agar [TSA]), volume of plate count agar and TSA per plate (5 and 10 ml), strength of overlaying violet red bile agar (VRBA, single to double strength), volume of sample per plate (0.1 to 3 ml), and time (15 min to 3 h) and temperature (25 to 40°C) of preincubation of plates (to allow repair of injured cells) before overlaying with VRBA (B. Ray and M. L. Speck, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, P12, p. 201). Adaptations of the method for the detection of coliforms from different types of semipreserved foods have been recommended (2). Among the variables, only the preincubation time and temperature of the plates before overlaying with VRBA were found to be important. Maximum enumeration was obtained when the preincubation was 1 h at 35°C or 2 h at 25°C; the use of 1 h at 25°C allowed enumeration of about 85% of the coliforms (unpublished data). Although other workers have reported no influence of preincubation time on coliform detection (4), our experience with repair of injured cells suggests that at least 1 h of preincubation at 25°C should be practiced, especially if samples contain high numbers of injured cells or strains that are relatively sensitive to bile salts. We found that effective and practical enumeration of both injured and uninjured coliforms in semipreserved foods could be accomplished with a pour-overlay method. This procedure consists of pour plating the initial portion of the sample with a nutritionally complete medium, such as TSA, followed by 1 h of incubation at room temperature (25°C) for repair of injured cells; an overlay is then poured, using a selective agar medium, such as VRBA. The selective components of VRBA diffuse through the TSA and create an environment in which both repaired and uninjured coliforms can multiply and form typical colonies. Because semipreserved foods produced under sanitary conditions are expected to contain relatively small numbers of coliforms, most coliform standards for foods are less than 100/g; for meaningful results, the amount of the sample to be tested must be relatively large, i.e., 0.1 to 1.0 g or 1 to 10 ml. The pour-overlay method has advantages over the surface-overlay method because more sample can be added per plate. We, therefore, explored the use of a pour-overlay method for the detection of coliforms in different commercially produced dairy products and in other types of semipreserved foods. This note reports some of these findings.

Different types of dairy products were obtained from local grocery stores, from our departmental processing plant, and from the Food and Drug Testing Laboratory of the North Carolina Department of Agriculture. The samples were analyzed on the same day that they were obtained and before their indicated expiration date. In general, sampling, preparation of different types of samples, and plating on VRBA were done according to standard procedures (1). All samples, except liquid milk, were diluted 1:10, and a 10-ml portion (equivalent to 1 g of sample) was plated in three plates; for liquid milk, 1-ml portions were plated in duplicate. In the standard procedure (1), all plates were first poured with VRBA (at 45°C), allowed to stand at room temperature for about 15 min to solidify, and then overlaid with about 5 ml of VRBA. In the

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"repair-detection" method, a duplicate set of plates, containing a portion of sample as noted above, were first pour plated with 5 ml of TSA per plate and left at room temperature for 1 h to allow repair of the injured coliforms present in the sample. These plates were then overlaid with 10 to 12 ml of VRBA per plate to induce a selective environment. After solidification of the medium, the plates were incubated at 35°C for 24 h. All colonies showing typical characteristics (1) were counted as coliforms. For confirmation, up to five representative colonies from each sample for each plating method were transferred to brilliant green lactose bile (BGLB) broth tubes, incubated at 35°C for up to 48 h, and examined for gas formation. Any colony that formed gas in a BGLB tube was counted as a confirmed coliform; these data were used to determine the percentages of colonies giving a confirmed coliform test in each plating method. The colonies that did not form gas in a BGLB tube were examined further in an effort to identify them (5).

The results of this study, consisting of a total of 622 samples including 202 of ice cream and 3 ice milk, are shown in Table 1. Coliforms were found most frequently in ice cream; about 62% of the samples were positive. In contrast, coliforms could not be detected from any unfrozen yogurt and nonfat dry milk samples. Average coliform populations measured by the VRBA (standard) and TSA/VRBA (repair-detection) methods varied with the type of samples; largest differences in counts were observed in soft cheese, ice milk, and buttermilk. With the exception of cottage cheese, more coliforms were detected by the repair-detection method than by the standard method, indicating the presence of injured coliforms in these products and the effectiveness of the repair-detection method for their enumeration. Coliforms from both plating methods gave high confirmation on BGLB, indicating that the repair-detection method lost no selectivity for coliforms.

A limited number of colonies that failed to produce gas in BGLB in 48 h at 35°C were purified and identified (5) as *Enterobacter agglomerans, E. hafniae,* and *Erwinia* sp. These colonies, isolated from both VRBA and TSA/VRBA plates, were generally small; with little practice, they were differentiated from the typical coliform colonies on the plates.

The suitability of the repair-detection procedure for coliforms in various types of commercial semipreserved foods was tested. Each sample, after being blended in a 1:10 ratio with 0.1% peptone water, was tested by a nine-tube, most-probable-number (MPN) method, by the VRBA

TABLE 1. *Coliform enumeration in dairy products by standard and repair-detection procedures*

Type of sample[a]	No. tested/no. positive[b]	% Positive	Avg coliform/g on[c]:		% Increase[d] on TSA/VRBA	% Confirmed[e] in BG-B from:	
			VRBA	TSA/VRBA		VRBA	TSA/VRBA
Liquid milk	197/48	24.4	8	10	20	95	95
Cream	40/2	5.0	22	26	18	75	100
Buttermilk	46/7	15.4	17	23	35	94	96
Yogurt	32/0	0					
Cottage cheese	45/5	11.1	6	6	0	100	100
Soft cheese	24/3	12.5	13	20	54	100	100
Nonfat dry milk	25/0	0					
Ice milk	3/1	33.3	86	125	45	100	100
Sherbet	2/1	50.0	1	1	0	100	100
Frozen yogurt	8/1	12.5	0	1			100
Ice cream	202/125	61.9	15	18	20	94	94
Avg on total sample	624/193	30.9	13	16	20	95	98

[a] Liquid milk includes regular, low-fat, skim, flavored, chocolate, and acidophilus milk. Cream includes cream and half-and-half. Ice cream samples included different flavors, sizes, and novelties.

[b] Any sample from which coliforms were detected and counted as positive.

[c] The samples that gave a positive count in a 10^0 dilution were included in this calculation. A total of 20 samples, mostly from ice cream, were too numerous to count in 10^0 to 10^1 dilutions and were not included in this calculation. A total of 22 samples, many from liquid milk, gave positive counts only in TSA/VRBA and not on VRBA; however, in 8 samples, counts were detected only on VRBA and not on TSA/VRBA. Also, in 10 samples, counts were more than 10/g by TSA/VRBA and less than 10/g by VRBA; in 4 samples, the reverse was true.

[d] Only the samples that had low coliform counts were considered (mostly less than or slightly higher than 10/g). Thus, the average percent increase was not very high; on individual samples, TSA/VRBA detected 100 to 200% or more coliforms than were detected on VRBA.

[e] A total of 635 colonies from TSA/VRBA and 555 colonies from VRBA plates were examined.

pour method, and by the TSA/VRBA pour method with the following modification: only a 0.5-ml volume was used per plate in the VRBA and TSA/VRBA methods, and the repair step was at 35°C for 2 h before overlaying with VRBA. The MPN procedure involved using lauryl sulfate tryptose broth, followed by confirmation in BGLB (2, 3). The results are shown in Table 2. In general, about 80% of the colonies enumerated as coliforms on VRBA and TSA/VRBA were confirmed as coliforms. However, average numbers of confirmed coliforms detected by TSA/VRBA far exceeded the numbers detected by the MPN method or by VRBA plating. This indicated that injured coliforms were present in these foods. The injury could have resulted from the different types of stress imposed by processing and storage methods, as well as during transportation of the samples in dry ice from the collection point to the testing laboratory. Cells in such a condition would not be detected by the recommended MPN method. Selected colonies that resembled coliforms on VRBA and TSA/VRBA, but failed to confirm on BGLB, were tested biochemically (5). These were identified as lactose-nonfermentating *Enterobacter aerogenes, E. cloacae, E. agglomerans, Citrobacter* sp., *Klebsiella* sp., *Erwinia* sp., *Proteus* sp., and *Alcaligenes* sp. All, except the last two groups, were positive for β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside positive). How these *o*-nitrophenyl-β-D-galactopyranoside-negative isolates can produce red to pink colonies on VRBA or TSA/VRBA is not known; food materials possibly provide utilizable carbohydrates from which enough acid is formed to produce the characteristic colonies. The noncoliform colonies were detected more frequently in seafoods and in ground beef than in other kinds of semipreserved foods tested in this study.

TABLE 2. *Efficiency of three methods for the detection of coliforms from different semipreserved foods*

Method	Avg coliform counts per g[a]		% BGLB positive[b]	
	Frozen seafoods[c]	Frozen and refrigerated products[c]	Frozen seafoods	Frozen and refrigerated products
MPN	218	851	100	100
Pour plating				
VRBA	166	533	80	79
TSA/VRBA	338	1,710	78	83

[a] Computed for each method by dividing the total coliform counts per gram by the number of samples tested.

[b] As only the positive BGLB tubes were considered as confirmed for coliforms in the MPN method, the confirmation value was 100%.

[c] A total of 30 frozen seafoods and 39 frozen and refrigerated products consisting of the following were used: 5 fish fillets, 3 stuffed flounders, 4 deviled crabs, 2 crab cakes, 12 shrimp, 4 oysters, 1 TV dinner, 1 chicken à la king, 1 Salisbury steak, 3 hash browns, 1 onion ring, 8 beef patties, 6 ground beef, 3 vegetable protein milk, 1 vegetable, 3 sausages, 2 franks, 2 bacon, 3 egg salad, and 4 chicken salad. A large number of samples were screened by plating 1 g equivalent on VRBA to check for the presence of coliforms. Only those containing coliforms were included in this study.

The repair-detection method for coliforms appears to be less effective for all types of semipreserved foods than for dairy products; however, it is definitely more accurate than the currently recommended MPN method for enumerating coliforms.

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