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Comparison of Various Plating Procedures for the Detection and Enumeration of Coliforms in Ice Cream and Ice Milk

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ABSTRACT

Eight plating procedures for the detection and enumeration of coliforms in ice cream and nonfat frozen dessert were compared. The procedures were: (i) direct plating of 1 ml, (ii) 2 ml, or (iii) 3 ml of product with violet red bile agar (VRBA), (iv) direct plating of 5 ml of product with VRBA in a large petri dish, (v) plating 10 ml of a 1:10 dilution of the product across three plates poured with VRBA, (vi) plating 1 ml of a 1:10 dilution with VRBA, and (vii) plating 1 ml of a 1:10 dilution to Petrifilm E. coli Count (PEC) plates incubated for 24 and (viii) 48 h. Three samples of ice cream (high-fat chocolate, high-fat vanilla, and high-fat strawberry) and three samples of frozen dessert (fat-free chocolate, fat-free vanilla, and fat-free strawberry) were selected as representative products and were inoculated with coliforms for use in the study. The data indicated that direct plating of ice cream or frozen dessert was less reliable than plating a diluted product for detection and enumeration of coliforms. Results of platings with VRBA and PEC of 1-ml portions of a 1:10 dilution were closely related to the results of the Standard Methods agar procedure of plating 10 ml of a 1:10 dilution for detection and enumeration of coliforms.

According to Standard Methods for the Examination of Dairy Products (SMEDP) *(4)*, the agar method for enumeration of coliforms in ice cream is to make a 1:10 dilution of the product by combining 11 g of product with 99 ml of sterile dilution water. The dilution is homogenized by shaking. Ten ml of this homogenate is distributed equally to three 100-mm petri dishes, to which tempered violet red bile agar (VRBA) is then added. Once the agar has hardened, 3-4 ml of VRBA is added as an overlay to restrict the growth of colonies on the agar surface. After incubation at 32°C for 24 h, representative presumptive coliforms are cultured in brilliant green bile (BGB) broth incubated at 32°C for 48 h for coliform confirmation. If high counts (>450 CFU per g) are consistently encountered in the ice cream samples, it is recommended that 1 ml of the 1:10 homogenate be pipetted to a fourth plate. Whether plating 10 ml to three petri dishes or 1 ml to a single petri dish, all platings are performed in duplicate.

The SMEDP procedure and Association of Official Analytical Chemists (A.O.A.C.) method *(1)* for using Petrifilm plates to enumerate coliforms in ice cream requires preparation of a 2:3 dilution of ice cream, and plating 0.5 ml of this dilution onto each of three prehydrated coliform count or Petrifilm E. coli Count plates. One can also plate any higher dilution, such as 1:10, onto the Petrifilm plates and attain comparable results.

In an effort to find a more convenient method or to increase perceived sensitivity, laboratories may plate one or more ml of ice cream directly to an agar plate with no dilution step. This is not, however, an approved method and may not give accurate results.

This study compared the SMEDP agar method and dry film and agar dilution methods to direct product (no dilution) plating methods. The methods compared were: (i) direct plating 1 ml, (ii) 2 ml, or (iii) 3 ml of product with VRBA in a 100 mm petri dish, (iv) direct plating 5 ml of product with VRBA in a 150 mm petri dish, (v) plating 10 ml of 1:10 dilution to VRBA in 3 petri dishes, (vi) plating 1 ml of 1:10 dilution to VRBA, (vii) plating 1 ml of a 1:10 dilution of product to Petrifilm E. coli Count (PEC) plates incubated for 24 and (viii) 48 h.

MATERIALS AND METHODS

Test products

The six ice cream lots used for inoculation in this study were purchased from a local supermarket and screened for the presence of coliforms using the SMEDP standard agar pour plate procedure. All products had a coliform count of <1 CFU per g. A single product lot of each type was used.

Test organisms

In an effort to insure that the coliforms were typical dairy product isolates, raw milk was selected as a source of the coliform cultures. Twenty raw milk samples obtained from a local dairy were screened using SMEDP standard agar pour plate procedure to determine the level of coliforms. Some of the milk samples were incubated in a 35°C water bath for 2 h to increase the coliform populations prior to inoculation.

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Inoculation of test samples

Twenty-one 50-g samples of each type of ice cream were aseptically transferred to individual sterile jars. The samples were allowed to soften at 4°C for 1-2 h. Each of the 20 samples of raw milk was used to inoculate one jar of each type of ice cream. The raw milk was added to obtain a level of 10-100 coliforms per g of ice cream. After inoculation, the samples were shaken to insure distribution of the inoculum and were refrozen at -20°C for 24 h to simulate temperature conditions to which the organisms would be exposed. The 21st sample of each product served as an uninoculated control and was subjected to the same conditions as the inoculated samples.

Microbiological analyses

Samples were thawed at 4°C for 1-2 h prior to analysis. Direct platings with VRBA (BBL, Cockeysville, MD) were performed by pipetting 1, 2, and 3 ml to sterile 100-mm petri dishes, or 5 ml to sterile 150-mm petri dishes, before addition of 10-15 ml tempered VRBA (for 1, 2, and 3 ml) and 20-25 ml tempered VRBA (for 5 ml). Sample homogenates were prepared by addition of 11 g of sample to 99 ml of warmed (40°C) sterile Butterfield's phosphate-buffered dilution water (Bacteriologial Analytical Manual, 6th ed., AOAC, Arlington, VA, 1984, pH 6.8-7.0, EM Science, Cherry Hill, NJ). The SMEDP procedure was performed by pipetting 10 ml of the homogenate across three sterile 100-mm petri dishes and 1 ml to a single sterile petri dish to which tempered VRBA was added and mixed with the sample, allowed to solidify, and overlaid with VRBA. Dilution platings to PEC (3M, St. Paul, MN) were performed by pipetting 1 ml of the homogenate to PEC. Platings for all methods tested were performed in duplicate. All plates were incubated at 32°C and were read after 24 h. When applicable, three typical colonies from each plate were confirmed in BGB (BBL, Cockeysville, MD) incubated at 32°C for 48 h. PEC plates were incubated an additional 24 h and were read a second time. A schematic diagram of the platings is presented in Fig. 1.

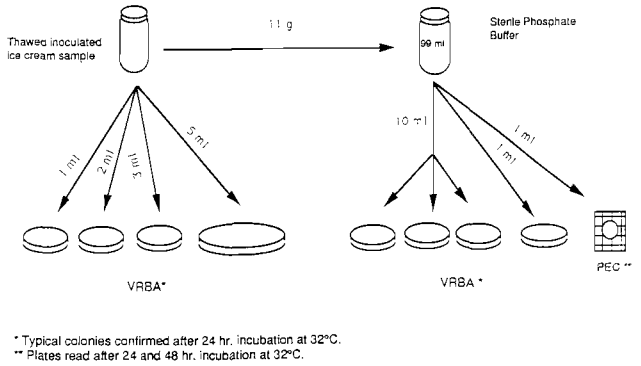

Figure 1. *Schematic representation of microbiological analyses.*

Statistical analyses

Coliform counts were first converted to $\log_{10}$ counts to more nearly match the underlying assumption of a normal distribution. Analysis of variance and Tukey's Honestly Significant Difference (HSD) Method were used to compare the mean log counts for the methods used in this study. In the table, means in the same row which are not followed by the same letter are significantly different ($P<0.05$). Also, the proportion of samples where coliforms were detected was calculated for each food and method. These proportions were compared by analysis of variance and Tukey's HSD method (*5*).

RESULTS

No coliform colonies were observed on any of the plates from the uninoculated control samples.

Comparison of mean log counts

The mean log counts and the standard deviations for the analyses are presented in Table 1. None of the high-fat chocolate and six of the high-fat vanilla samples had coliform levels that were detected by all methods for each sample. Comparison of means was not done on these samples. The results suggested that the direct plating methods were similar to each other, and that the dilution plating methods were similar to each other. The mean log counts for the dilution plating methods were significantly higher than for the direct plating methods. This suggests, with few exceptions, that the dilution plating methods recover higher numbers of organisms than direct product plating.

Comparison of frequency detection

The number of samples from which coliforms were detected from each product and method is presented in Table 2. These results indicated that there were no significant differences between methods for the high-fat vanilla and strawberry ice creams and the fat-free chocolate and strawberry frozen desserts. Differences were noted with fat-free chocolate if 3 or 5 ml were added directly to agar plates. No statistical differences were noted between 24 and 48 h reading of PEC plates.

DISCUSSION

Deviations from standard procedures may result in under- or overestimation of coliforms in ice cream for various reasons:

a. False-positive results may occur if organisms other than coliforms ferment sugar in the ice cream (*4*). This effect is more likely with direct, undiluted product plating because the sugars are not diluted. Confirmation of typical coliform colonies in BGB is necessary.

b. The ice cream may contain ingredients, such as chocolate, that inhibit growth. Coliform colonies could have an atypical appearance in these ice creams, and cause false-negative results.

c. Inaccurately low coliform counts may be obtained when excess product on the plates causes overcrowding of colonies resulting in atypically sized colonies.

d. Coliform counts may be underestimated if pipets are used to transfer undiluted sample because ice cream adheres to the interior of the pipet. The viscosity of the ice cream prevents accurate measurement using a pipet. Weighing the product eliminates the problem. With subsequent dilutions, pipets may be used to transfer samples because the dilution step decreases the viscosity and less of the sample adheres to the pipet.

In addition, the flora of the sample may affect what happens in the analyses. Although overall differences were not great, we saw all the above in this study with certain samples.

TABLE 1. *Mean log counts (CFU/g or CFU/ml) and standard deviations for coliforms in ice cream.**

		Mean log counts and standard deviations**																					
		Direct to VRBA (CFU/ml)										1:10 Dilution (CFU/g)											
Product	N***	1 ml			2 ml			3 ml			5 ml			SMEDP			1 ml VRBA			24 h PEC			48 h PEC		
High-fat strawberry	18	1.76	ab	(0.59)	1.75	ab	(0.59)	1.64	a	(0.64)	1.82	ab	(0.74)	2.02	bc	(0.96)	2.17	c	(0.65)	1.98	abc	(0.62)	2.02	bc	(0.59)
Fat-free chocolate	12	1.05	ab	(0.79)	0.63	a	(0.83)	0.86	a	(0.75)	0.79	a	(0.95)	1.44	b	(0.75)	1.44	b	(0.67)	1.38	b	(0.69)	1.48	b	(0.63)
Fat-free vanilla	12	1.24	a	(0.79)	1.30	a	(0.72)	1.26	a	(0.78)	1.33	ab	(0.86)	1.73	b	(0.76)	1.65	ab	(0.91)	1.62	ab	(0.68)	1.67	b	(0.64)
Fat-free strawberry	13	1.40	a	(0.95)	1.33	a	(0.94)	1.32	a	(0.90)	1.22	a	(0.75)	1.96	b	(0.73)	1.94	b	(0.91)	1.85	b	(0.75)	1.95	b	(0.68)

* None of the high-fat chocolate and six of the high-fat vanilla samples had coliform levels that were detected by all methods. Comparison of means was not done on these samples.
** Values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$). Values in parenthesis indicate standard deviations.
*** N = Number of samples compared.

TABLE 2. *Frequency of coliforms detected by each test procedure.**

		Direct to VRBA (CFU/ml)								1:10 Dilution (CFU/g)							
Product	N**	1 ml		2 ml		3 ml		5 ml		SMEDP		1 ml VRBA		24 h PEC		48 h PEC	
High-fat chocolate	20	14	a	11	ab	3	c	5	bc	14	a	15	a	12	ab	15	a
High-fat vanilla	20	13	a	16	a	13	a	18	a	13	a	11	a	14	a	16	a
High-fat strawberry	20	19	a	19	a	19	a	20	a	19	a	18	a	20	a	20	a
Fat-free chocolate	20	20	a	20	a	18	a	18	a	19	a	16	a	18	a	20	a
Fat-free vanilla	20	20	a	20	a	19	ab	19	ab	19	ab	15	b	18	ab	18	ab
Fat-free strawberry	20	18	a	19	a	17	a	17	a	18	a	16	a	18	a	18	a

* Values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$).
** N = Number of samples compared.

In this study, more false-negative results than false-positive results were observed. Greater than 50% of the plates generated by direct plating of 3- and 5-ml portions of high-fat chocolate ice cream to VRBA did not have typical coliform colonies. For the other five products, 6% of the plates from direct plating did not have typical coliforms. Only 1% of the plates from the dilution platings did not have typical coliforms. Confirmation in BGB of atypical colonies indicated that the colonies were coliforms, thus, suggesting that the quantity of product plated affects the formation of typical coliform colonies.

Dilution plating to PEC offers several advantages over direct plating and the SMEDP procedure for enumeration of coliforms. The procedure requires less time than the SMEDP procedure; a confirmed test using the SMEDP procedure requires 3 d. PEC plates are incubated for 24 h. Even if a 48 h incubation time is used, time is still saved because colony confirmation in BGB is not specified in the AOAC method (*2,3*). Based on the data obtained for 5 of the 6 products tested, the PEC methods, with few exceptions, had higher confirmation rates (94-100%) than any of the other methods. Dilution plating to PEC also uses a more representative sample than the direct plating to VRBA because 11 g of product is homogenized rather than 1 g of product plated directly. Dilution plating decreased the risk of false-positive results because of unwanted fermentation of sugar contributed by the ice cream to the plate. Although the possible need for a 48 h incubation of PEC was investigated, no statistically significant differences were found between the 24 h results and the 48 h results.

CONCLUSION

In general, dilution plating of ice cream produced higher counts and fewer false negatives than direct plating.

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