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Conventional versus the petrifilm method for the enumeration of different types of bacteria[1]

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ABSTRACT

Performance of Petrifilm VRB and SM plates to enumerate different types of bacterial populations was evaluated. A total of 100 Petrifilms and 100 regular Petri plates were tested with each of 3 different bacterial populations in 4 experiments. The Petrifilm system was superior to the conventional pour plate methods, or at least not statistically different, in enumerating *Escherichia coli* (coliform) and a mixture of *E. coli*, *Staphylococcus aureus* and *Serratia marcescens* (total aerobic plate count). The Petrifilm method was superior to conventional plate counts in enumerating *Pseudomonas aeruginosa* (psychrotrophic plate count), when both incubations were at 21°-22° C for 96 h. If sustained in further experimentation, this last observation would mean that the Petrifilm SM system could be successfully used to detect bacterial populations in addition to those for which the system was originally intended.

RESUMEN

Método corriente y el de Petrifilm para enumerar diferentes tipos de bacterias.

Se evaluó el comportamiento de las placas "Petrifilm VRB" (detección de coliformes) y SM (detección de conteo total) para enumerar diferentes tipos de bacterias. Se llevaron a cabo 4 experimentos comparativos, en los que se utilizaron un total de 100 placas "Petrifilm" y 100 placas Petri corrientes en cada una de 3 muestras de diferentes bacterias. El sistema "Petrifilm" demostró ser superior o, al menos, no estadísticamente diferente al corriente al enumerar *Escherichia coli* (coliforme) y una mezcla de *E. coli*, *Stephylococcus aureus* y *Serratia marcescens* (conteo total aeróbico). Cuando se incubaron a entre 21° y 22° C. por 96 horas, el sistema "Petrifilm" demostró ser mejor para la enumeración de *Pseudomonas aeruginosa* (conteo psicrotrófico). Si esto se repite en subsiguiente experimentación, significaría que el sistema "Petrifilm SM" podría usarse para detectar y enumerar la presencia de bacterias distintas a las que originalmente se pretendía cuantificar cuando se diseñó el sistema.

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INTRODUCTION

The ever increasing need to improve efficiency in the microbiological analysis of foods, milk and water has led to the development of innovative artifacts, equipment and techniques to serve this purpose. An example of this is Petrifilm plates.

This system, developed by the 3M Company, reduces the time and effort required to enumerate bacteria as compared to the conventional poured plate method. The system has been evaluated for milk (2,3,5), fresh ground beef (6) and food-contact surfaces (4). In all these studies the Petrifilm method has been described as a suitable alternative to the conventional method.

The purpose of this study was to evaluate the performance of the Petrifilm method in enumerating bacteria from aqueous suspensions of cultures of different bacteria. This evaluation was done to assess the possibility of using the Petrifilm method for monitoring the microbiological quality of water samples in food processing plants.

MATERIALS AND METHODS

Petrifilm SM Plates (developed for determining total aerobic microbial populations) and VRB Plates (for coliform determination), were obtained locally from 3M Puerto Rico, Inc., San Juan, P.R. Preserved cultures of *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 8100, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were reconstituted and cultured in Trypic Soy Broth (TSB; Difco Laboratories, Detroit, Michigan)[3] at 37° C for 24 h. Each culture was then transferred to Tryptic Soy Agar slants (TSA; Difco) and incubated at 37° C for 24 h. They were subsequently stored at 4° C. These slants comprised the primary stock cultures used to perform the experiments. Gram stains and biochemical tests were also performed to determine the purity of each culture.

For each of the four identical experiments, we prepared fresh bacterial cultures by subculturing each primary stock culture in Nutrient Broth tubes (NB; Difco) and incubating each at 35° C for 24 h. We prepared additional TSA slants from each NB as previously described to be used as the stock cultures of the next experiment. The working cultures were prepared after each NB tube was incubated. We prepared cultures of *E. coli* and *P. aeruginosa* by adding 1 ml of the NB culture to 15 ml fresh sterile NB. A mixed culture composed of *S. marcescens*, *E. coli* and *S. aureus* was prepared by adding 2 ml of each individual NB culture

[3]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment of materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

to 15 ml fresh sterile NB. From these 3 cultures, we prepared appropriate 10-fold serial dilutions in 0.1% sterile peptone water and pour-plated them to obtain a dilution that gave between 15 and 150 colony-forming units (CFU) per ml. The conventional methods used for enumerating *E. coli* (Coliform Group, Solid Medium Method), the mixed culture (Total Aerobic Plate Count) and *P. aeruginosa* (Psychrotrophic Bacteria in Meat and Poultry Products) were described by Speck (7). All working cultures were stored at 4° C until their cell concentration was determined. This procedure was followed in its entirety in all 4 experiments.

Once the above information was obtained, cultures in each experiment were counted by 2 methods: conventional pour plates and Petrifilm plates. We enumerated the *E. coli* suspensions by using Violet Red Bile Agar (VRBA; Difco) incubated at 350° C for 24 h and Petrifilm VR13 plates incubated at 32° C for 24 h. The mixed culture suspensions were enumerated by means of Plate Count Agar (PCA; Difco) incubated at 35° C for 48 h and petrifilm SM plates incubated at 32° C for 48 h; and the *P. aeruginosa* suspensions by using PCA and Petrifilm plates, all incubated at 21°-22° C for 96 h. We used a total of 25 conventional Petri plates and 25 Petrifilm plates in each experiment.

After the corresponding incubation period, bacterial populations were counted in the Petri and Petrifilm plates. Using a Student's t-test, we compared the mean values which were computed for each set of 25 counts.

RESULTS AND DISCUSSION

Results are presented in table 1. For enumerating *E. coli*, the Petrifilm VRB plates were better than conventional VRBA pour plates in 2 experiments, and were not significantly different ($P > 0.05$) in the other two. These observations were consistent with those of Nelson et al. (5). They reported that although there was a significant difference between methods, Petrifilm VRB was a viable alternative to plating on VRBA, and that both methods could be considered microbiologically equivalent. In the present work, *E. coli* counts on Petrifilm VRB were lower than in the VRBA plates in 2 experiments, but these differences were not statistically significant. In the other 2 experiments, however, the counts on Petrifilm VRB were significantly higher ($P \leq 0.05$) than in the VRBA plates.

The total aerobic plate counts obtained from the mixture of microorganisms (*E. coli*, *S. aureus* and *S. marcescens*) followed the same trend as the *E. coli* counts; that is, the Petrifilm SM plates were superior to the PCA in 2 experiments and were not significantly different ($P > 0.05$) in the other two. However, a difference was observed in the experiments in which no statistical differences were found: the Petrifilm SM counts were higher than those obtained with conventional PCA pour plates.

TABLE 1.—Comparison of the average colony forming units (CFU) per ml of different bacterial suspensions determined by two methodologies

Bacterial group	Experiment	Mean Values (CFU)[1]	
		Conventional	Petrifilm
Coliform[2] (*E. coli.*)	1	70.4 a[3]	67.8 a
	2	111.3	124.1
	3	103.7	143.2
	4	99.4 b	95.6 b
Total Aerobic[4] Plate Count (*E. coli., S. aureus, S. marcescens*)	1	49.6	54.8
	2	116.4 c	121.4 c
	3	22.2 d	22.6 d
	4	64.6	88.0
Psychrotrophic[5] Plate Count (*P. aeruginosa*)	1	14.6	17.2
	2	101.3	128.4
	3	75.6	91.2
	4	17.4	28.7

[1]Each value is the computed mean of 25 Petri plates (Conventional) or 25 Petrifilms per experiment.

[2]Conventional method: VRBA poured plates incubated at 35° C for 24 hours. Petrifilm method: VRB plates incubated at 32° C for 24 hours.

[3]Means in columns followed by the same letter do not differ significantly at ($P > 0.05$).

[4] Conventional method: PCA poured plates incubated at 35° C for 48 hours. Petrifilm method: SM plates incubated at 32° C for 48 hours.

[5]Conventional method: PCA poured plates incubated at 21°-22° C for 96 hours. Petrifilm method: SM plates incubated at 21°-22° C for 96 hours.

This finding contrasts with the observations of Ginn et al. (2) and of McGoldrick et al. (4).

The most interesting observations were the results obtained in the psychrotropic plate count (*P. aeruginosa*). Both the Petrifilm and the PCA plates were incubated at 21°-22° C for 96 h. In all 4 experiments, the counts obtained with the Petrifilm were significantly higher ($P \leq 0.05$) than the ones obtained with the conventional PCA plate method. This observation, if sustained in further experimentation, would mean that the Petrifilm SM system could be used effectively under incubation conditions in addition to those recommended by the manufacturer, that is, to detect and enumerate bacterial populations for which the system was not originally designed.

Psychrotrophic bacteria, particularly *Pseudomonas*, are commonly found in water and can contaminate and spoil refrigerated foods (1, 7). The detection and enumeration of psychrotrophic bacteria is very important because their presence in foods in large numbers creates a high potential for spoilage during extended refrigeration. The presence and quantity of psychrotrophic bacteria in water is indicative of the contami-

nation potential of such water, particularly for foods that are stored at low temperatures either raw or only partially cooked.

In conclusion, the results obtained in this study suggest that the Petrifilm method is better than or at least as good as conventional plate count methods used to enumerate coliforms (*E. coli*) and total aerobic bacteria. The findings also suggest that the Petrifilm method is better than conventional plate count methods for enumerating psychrotrophic bacteria (*P. aeruginosa*); thus giving a new dimension in the scope of application for Petrifilm SM plates.

LITERATURE CITED

1. Frazier, W. C. and D. C. Westhoff, 1988. Food Microbiology, 4th ed., McGraw-Hill Book Co., New York.
2. Ginn, R. E., V. S. Packard and T. L. Fox, 1984. Evaluation of the 3M dry medium culture plate (Petrifilm SM) method for determining numbers of bacteria in raw milk. *J. Food Prot.* 47: 753–55.
3. McAllister, J. S., M. S. Ramos and T. L. Fox, 1987. Evaluation of the 3M dry medium culture plate (Petrifilm SM) method for enumerating bacteria in processed fluid milk samples. *Dairy Food Sanit.* 7: 632–35.
4. McGoldrick, K. F., T. L. Fox and J. S. McAllister, 1986. Evaluation of a dry medium for detecting contamination on surfaces. *Food Technol.* 40 (4): 77–80.
5. Nelson, C. L., T. L. Fox and F. F. Busta, 1984. Evaluation of dry medium film (Petrifilm VRB) for coliform enumeration. *J. Food Prot.* 47: 520–25.
6. Smith, L. B., T. L. Fox and F. F. Busta, 1985. Comparison of a dry medium culture plate (Petrifilm SM plates) method to the aerobic plate count method for enumeration of mesophilic colony-forming units in fresh ground beef. *J. Food Prot.* 48: 1044–045.
7. Speck, M. L., Ed., 1976. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D. C.