Comparison of Dry Medium Culture Plates for Mesophilic Aerobic Bacteria in Milk, Ice Cream, Ham, and Codfish Fillet Products

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ABSTRACT: This study was performed to compare the performance of Sanita-Kun dry medium culture plate with those of traditional culture medium and Petrifilm dry medium culture plate for the enumeration of the mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet. Mesophilic aerobic bacteria were comparatively evaluated in milk, ice cream, ham, and codfish fillet using Sanita-Kun aerobic count (SAC), Petrifilm aerobic count (PAC), and traditional plate count agar (PCA) media. According to the results, all methods showed high correlations of 0.989~1.000 and no significant differences were observed for enumerating the mesophilic aerobic bacteria in the tested food products. SAC method was easier to perform and count colonies efficiently as compared to the PCA and PAC methods. Therefore, we concluded that the SAC method offers an acceptable alternative to the PCA and PAC methods for counting the mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet products.

Keywords: bacteria, mesophilic, Petrifilm, plate count agar, Sanita-Kun

INTRODUCTION

Food spoilage by microorganisms results in economic loss to consumers, processors, and producers (1). Predicting shelf life and determining the spoilage status of foods accurately can reduce microbial spoilage of foods (1). Mesophilic aerobic bacteria can be used as indicators for the determination of shelf life and spoilage status of foods, and they are useful to evaluate the sanitary quality of foods (1,2) because their presence implies contamination by pathogens in food (3,4).

Incidence of food poisoning in livestock and fishery products is increasing; for this reason, safety control of livestock and fishery products is very important. Direct analysis of pathogens in food is difficult and costly (5). Hence, analysis of mesophilic aerobic bacteria as an indicator can be an alternative to evaluate the sanitary quality of foods (1,2).

Methods for the enumeration of the mesophilic aerobic bacteria have commonly used a traditional standard plate count method (5). However, this method has several disadvantages, such as time-consuming, labor-intensive, bulky waste, and cumbersome (6,7). In order to eliminate these shortcomings, dry medium culture plate methods, such as Petrifilm and Sanita-Kun dry medium

culture plates, could be alternatives to the traditional culture method (7-9).

Petrifilm dry medium culture plate consists of a cover film and a bottom film. The cover film contains adhesive, a cold water-soluble gelling agent, and 2,3,5-triphenyltetrazolium chloride (TTC) (10). Here, TTC is reduced by bacterial metabolism to produce a visible red dye (11). The bottom film contains a cold water-soluble gelling agent and dried nutrients (10). In comparison, Sanita-Kun dry medium culture plate consists of a transparent cover film, an adhesive sheet, and a layer of non-woven fabric that includes water-soluble polymers containing nutrients for microorganisms as well as TTC (7,12).

In the food industry, rapid and accurate inspection is urgent as it must consider both mass production and food quality concerns at the same time. Although many reports have compared Petrifilm dry medium culture plate method to traditional culture medium (8,13-15), comparison studies between dry medium culture plates have been limited. Therefore, we compared the performance of both Petrifilm and Sanita-Kun dry media for enumerating mesophilic aerobic bacteria in order to evaluate acceptable alternative methods to traditional culture.

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MATERIALS AND METHODS

Media

Plate count agar (PCA, Becton, Dickinson and Co., Franklin Lakes, NJ, USA) medium was used in the traditional culture method. Sanita-Kun aerobic count (SAC, Chisso Corp., Tokyo, Japan) and Petrifilm aerobic count (PAC, 3M, Maplewood, MN, USA) were used as dry medium culture plates.

Pretreatment for food products

Milk, ice cream, ham, and codfish fillet were purchased from local markets in Daegu, Republic of Korea. Ham and codfish fillet were homogenized using a blender, whereas milk and ice cream were used directly without being homogenized. Each food sample (25 g) was placed into a stomacher bag (Labplas Inc., Ste-Julie, QC, Canada), after which 225 mL of 0.85% NaCl was added. The sample mixtures were stored at 35°C for different time periods (6, 8, 10, 12, and 15 h) to increase microbial counts for each sample. The sample mixtures were homogenized for 2 min in the stomacher (Bag mixer 400, Interscience, Saint-Nom-la-Bretèche, France) before plating on the media.

Traditional culture method

The traditional culture method used to assess the mesophilic aerobic bacteria was performed by standard plate counting method (16). Sample mixtures were serially diluted with sterile 0.85% NaCl solution. Then, 1 mL of each serially diluted sample was added to a Petridish plate, followed by the addition of 15 mL of PCA medium. The plate was then incubated for 24~48 h at 35±1°C to grow the mesophilic aerobic bacteria.

Dry medium culture plate method

The Sanita-Kun and Petrifilm dry medium culture plate methods were performed according to each manufacturer's instructions in order to assess the mesophilic aerobic bacteria (17,18). The transparent cover film of

each dry medium culture plate was opened, after which 1 mL of decimal dilution was added to the bottom film. After the transparent cover films were covered, PAC medium was pressed with a plastic spreader in order to spread the sample uniformly, whereas this step was not necessary for the SAC medium. These two dry medium culture plates were incubated for 24~48 h at 35±1°C. The appearance of red colonies was considered to be the mesophilic aerobic bacteria.

Statistical analysis

Experiments were conducted more than 3 times for each sample. Microbial counts were changed to log values for statistical analysis. Correlation coefficient, slope, and intercept values of the 3 methods (PCA, SAC, and PAC) were obtained to compare the performances of the methods. Analysis of variance was conducted at a significance level of $P<0.05$ using SPSS version 19 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

For the enumeration of mesophilic aerobic bacteria on PAC and SAC, we observed the liquefaction phenomenon on PAC medium and the diffuse colonies on both PAC and SAC media. The diffuse colonies could be *Bacillus* spp., which excretes agarase and disintegrates agar (18,19). Liquefaction phenomenon due to bacteria is a state in which the gel is only semi-solidified (7). Morita et al. (7) also observed liquefaction phenomenon on PAC and diffuse colonies on PAC and SAC. Especially, colonies on PAC were often broken and mobile during counting due to liquefaction phenomenon. On the other hand, SAC showed no problems in colony counting as the medium was not liquefied. Due to the liquefaction phenomenon on PAC, counting the mesophilic aerobic bacteria on SAC was easier than PAC.

The relationships among the media in all samples are shown in Fig. 1. The correlation coefficients between the

Fig. 1. Relationships between plate count agar, Sanita-Kun aerobic count, and Petrifilm aerobic count for enumerating the mesophilic aerobic bacteria in all food samples. PCA, plate count agar; SAC, Sanita-Kun aerobic count; PAC, Petrifilm aerobic count.

PCA and SAC, PCA and PAC, and PAC and SAC methods were 0.999, 0.995, and 0.996, respectively. All methods showed high correlations in all samples. The slope values of all graphs were near 1.0 (1.002~1.014). Based on these results, the 3 methods were highly correlated with each other for the determination of the mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet.

Table 1 shows the correlation between all media in each food. The correlation coefficients among all media were 0.989~1.000, and the slope values varied between 0.858 and 1.126. The correlation coefficients of the PCA and PAC methods for ice cream showed the highest correlation ($R^2=1.000$), whereas the lowest correlation ($R^2=0.989$) was observed for codfish fillet. Overall, the correlation coefficients of all methods were high for each food.

Table 2 shows the log values of the cell counts as mean $\pm$ SD (standard deviation) and result of statistical analysis. Microbial cell count numbers for mesophilic aerobic bacteria detection on the 3 media including PCA, SAC, and PAC did not reveal differences more than 0.2 log₁₀ CFU/mL counts (Table 2). Also, these media (PCA, SAC, and PAC) were not significantly different at $P<0.05$ for enumerating the mesophilic aerobic bacteria in each food.

Table 1. Comparison of plate count agar, Sanita-Kun aerobic count, and Petrifilm aerobic count for enumerating the mesophilic aerobic bacteria in each food

Sample	Comparison	Correlation coefficient	Slope	Intercept
Milk	PCA & SAC ¹⁾	0.999	1.034	-0.141
	PCA & PAC	0.999	1.012	0.030
	PAC & SAC	0.998	1.020	-0.163
Ice cream	PCA & SAC	0.999	1.032	-0.189
	PCA & PAC	1.000	1.023	-0.163
	PAC & SAC	0.999	1.008	-0.024
Ham	PCA & SAC	0.999	0.983	0.181
	PCA & PAC	0.999	1.006	-0.013
	PAC & SAC	0.998	0.977	0.199
Codfish fillet	PCA & SAC	0.999	0.977	0.256
	PCA & PAC	0.989	0.858	1.201
	PAC & SAC	0.990	1.126	-1.022

¹⁾PCA, plate count agar; SAC, Sanita-Kun aerobic count; PAC, Petrifilm aerobic count.

Table 2. Statistical analysis between media for enumerating the mesophilic aerobic bacteria (CFU/mL)

Sample	PCA ¹⁾	SAC	PAC
Milk	6.30 $\pm$ 3.04 ^{NS2)}	6.37 $\pm$ 3.15	6.41 $\pm$ 3.08
Ice cream	5.76 $\pm$ 2.22 ^{NS}	5.75 $\pm$ 2.29	5.73 $\pm$ 2.27
Ham	6.06 $\pm$ 2.10 ^{NS}	6.14 $\pm$ 2.07	6.09 $\pm$ 2.11
Codfish fillet	7.40 $\pm$ 1.82 ^{NS}	7.48 $\pm$ 1.78	7.55 $\pm$ 1.57

The values are log₁₀ bacteria counts/mL (mean $\pm$ SD).

¹⁾PCA, plate count agar; SAC, Sanita-Kun aerobic count; PAC, Petrifilm aerobic count.

²⁾Not significant.

Rosmini et al. (14) and Ginn et al. (20) reported that the correlation coefficient between the PCA and PAC methods in raw milk was as high as 0.92 and 0.950~0.954, respectively, concluding that the PAC method is suitable as an alternative to the PCA method for enumerating the mesophilic aerobic bacteria in raw milk (14,20). Ha (21) compared the PCA and PAC methods for the enumeration of the mesophilic aerobic bacteria in milk, ground beef, fishery surimi, Takju, and wheat flour. He reported that both the PCA and PAC methods were not significantly different at $P<0.05$ with the correlation coefficient values of 0.96~0.99 (21). Cho et al. (15) compared the PCA and PAC methods for the enumeration of the mesophilic aerobic bacteria in milk, ice cream, ham, and fishery products and reported that the PAC method can be used as an alternative to the PCA method based on correlation coefficients of 0.980~0.999 with no significant difference at $P<0.05$. Morita et al. (7) compared the SAC and PCA methods in 16 food samples; in their results, both the SAC and PCA methods were statistically different at $P<0.05$ and $P<0.01$ in food samples; however, the differences were small, and the correlation coefficient between the SAC and PCA methods was as high as 0.99 (7). Therefore, Morita et al. concluded that the SAC method is an acceptable alternative to the PCA method (7).

In the present study, all 3 methods (PCA, SAC, and PAC) showed high correlation coefficients (>0.98) and were not significantly different ($P<0.05$) for enumerating the mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet. Both the SAC and PAC have relative advantages over the traditional culture method, including ease of use, less space requirements, and little waste production (7,8,21). Regarding the comparison of 2 dry medium culture plates, the PAC method was relatively inconvenient compared with SAC since an additional step for spreading the sample with a plastic spreader was required. Even more, sample leakage occurred when the plastic spreader pressed the surface of the PAC medium strongly. Also, colonies on PAC were often broken and mobile during the count of colonies due to liquefaction phenomenon of the medium. In contrast, the SAC method was relatively easier to perform than the PAC method since it did not require an additional step using a plastic spreader, and the non-woven fabric of SAC absorbed the sample solution to prevent sample leakage (12). Therefore, we conclude that the SAC method is an acceptable alternative over the PCA and PAC methods for analyzing the mesophilic aerobic bacteria in milk, ice cream, ham, and codfish fillet products. The results obtained in this research could help users select an appropriate method with respect to efficiency, ease of use, and economical microbiological assay.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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