

Comparison of Culture Media, Simplate, and Petrifilm for Enumeration of Yeasts and Molds in Food

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ABSTRACT

The efficacy of three culture media, dichloran rose bengal chloramphenicol (DRBC), dichloran 18% glycerol agar (DG18), and potato dextrose agar (PDA) supplemented with two antibiotics, were compared with the Simplate and Petrifilm techniques for mold and yeast enumeration. The following foods were analyzed: corn meal, wheat flour, cassava flour, bread crumbs, whole meal, sliced bread, ground peanuts, mozzarella cheese, grated parmesan cheese, cheese rolls, orange juice, pineapple pulp, pineapple cake, and mushroom in conserve. Correlation coefficients of DRBC versus PDA and DG18 for recovering total mold and yeast counts from the composite of 14 foods indicated that the three media were generally equivalent. Correlation coefficients for Petrifilm versus culture media were acceptable, although not as good as between culture media. Correlation coefficients of Simplate versus DRBC, DG18, PDA, and Petrifilm for recovering total yeasts and molds from a composite of 11 foods demonstrated that there was no equivalence between the counts obtained by Simplate and other culture media and Petrifilm, with significant differences observed for the most foods analyzed.

Mold and yeast enumeration in food is useful to evaluate the quality of food and the degree of deterioration, and it is becoming an essential component for microbiological assurance programs. The development of culture media to isolate fungi from food has been improved, and new techniques for fungal detection in food have been developed.

When studies on comparison of methods to enumerate fungi from foods are being conducted, two points must be considered: (i) the standard culture media to be used as comparison and (ii) the food that will be studied. The International Commission on Food Mycology has recommended dichloran rose bengal chloramphenicol agar (DRBC) for food with high water activity (a_w) (6–9). For foods with reduced a_w (<0.95), dichloran 18% glycerol agar (DG18) is preferred because xerophilic fungi may be present (2, 5, 8, 9). However, most studies comparing techniques for fungal enumeration have used potato dextrose agar (PDA) or plate count agar supplemented with antibiotics as standard media.

The enumeration of yeast and mold has traditionally been one of the slowest laboratory analyses. The food industry needs a rapid and simple method for yeast and mold counts without compromising its sensitivity and efficacy. The alternative techniques that estimate total counts of fungal colonies are Petrifilm, Simplate, Iso Grid, and spiral plate count. Other techniques, such as detection of ergosterol, chitin, fungal volatiles, adenosine triphosphate, immunological methods, impedimetry, and conductometry, are restricted to some foods or only estimate fungal biomass (8).

Several studies have already compared the perfor-

mance of Petrifilm and culture media for yeast and mold counts (3, 4, 12, 15) and have found lower, equivalent, or higher counts compared with culture media, including acidified PDA, PDA plus chloramphenicol and chlortetracycline, chloramphenicol-supplemented plate count agar, oxytetracycline glucose yeast extract, DRBC, DG18, Sabouraud dextrose agar plus chloramphenicol and chlortetracycline, and malt extract agar plus chloramphenicol and chlortetracycline. Taniwaki et al. (12) evaluated the performance of Petrifilm YM for different flours and pulp fruits compared with six culture media. They concluded that although for some foods the total counts on Petrifilm were similar to or higher than those on culture media, visualization of colonies on Petrifilm was not satisfactory because it was often difficult to distinguish the colonies from food particles. Petrifilm also showed problems with spreading species of *Rhizopus*, *Mucor*, *Absidia*, *Syncephalastrum*, and *Trichoderma*. When one of these genera was present in the sample, the Petrifilm count was underestimated or not possible.

Spangenberg and Ingham (11) compared the techniques of Petrifilm and Iso Grid with PDA plus chlortetracycline and DRBC for enumeration of yeasts and molds in part-skim mozzarella cheese. In this study all methods were highly correlated ($r^2 \geq 0.96$).

The Simplate technique for molds and yeasts (SYM) is an alternative method to detect and quantify fungi in food. It uses multiple enzyme technology that correlates fungal enzyme activity to the presence of yeasts and molds. The medium emits fluorescence when enzyme substrates are metabolized by yeasts and molds. According to the manufacturer, the major advantage of the Simplate method is a relatively short incubation time and the ability to detect a wide range of cell numbers using a single test.

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TABLE 1. Total mean mold and yeast counts (\log_{10} CFU/g) obtained in several foods using PDA, DG18, DRBC, Petrifilm for yeasts and molds plates, and SYM^a

Samples	PDA plus (CFU/g)	DG18 (CFU/g)	DRBC (CFU/g)	Petrifilm (CFU/g)	SYM at 48 h	SYM at 72 h
Corn meal	3.50 (0.13)	3.54 (0.09)	3.52 (0.13)	2.24 (0.22)	3.59 (0.19)	3.59 (0.19)
Wheat flour	2.64 (0.47)	2.77 (0.12)	2.56 (0.32)	3.16 (0.26)	ND	3.33 (0.28)
Cassava flour	2.76 (0.30)	3.01 (0.21)	3.03 (0.20)	2.64 (0.30)	SM	SM
Bread crumbs	3.20 (0.24)	2.95 (0.14)	3.13 (0.09)	2.99 (0.05)	2.60 (0.31)	3.36 (0.49)
Whole meal	4.47 (0.31)	4.64 (0.06)	4.17 (0.20)	2.92 (0.15)	SM	SM
Sliced bread	3.66 (0.46)	3.69 (0.11)	3.75 (0.42)	3.66 (0.23)	ND	3.82 (0.34)
Ground peanuts	3.53 (0.16)	3.61 (0.09)	3.60 (0.11)	3.46 (0.21)	3.25 (0.06)	3.42 (0.09)
Mozzarella cheese	7.04 (0.20)	7.10 (0.20)	7.09 (0.15)	7.12 (0.17)	4.02 (0.27)	4.52 (0.19)
Grated parmesan cheese	7.54 (0.32)	7.64 (0.32)	7.44 (0.27)	7.29 (0.22)	ND	2.40 (2.56)
Cheese rolls	5.63 (0.37)	5.77 (0.45)	6.01 (0.28)	6.03 (0.29)	1.58 (1.99)	4.61 (0.34)
Orange juice	4.41 (0.17)	4.04 (0.13)	4.28 (0.19)	3.96 (0.11)	2.94 (0.19)	2.98 (0.17)
Pineapple pulp	4.98 (0.37)	5.09 (0.08)	5.05 (0.09)	4.79 (0.08)	4.42 (0.14)	>3.87 (0.00)
Pineapple cake	2.99 (0.34)	3.73 (0.28)	3.07 (0.46)	3.06 (0.29)	ND	2.82 (0.40)
Mushroom in conserve	6.13 (0.12)	6.15 (0.11)	6.04 (0.10)	6.26 (0.41)	4.85 (0.29)	5.94 (0.20)

^a Mean of 7 to 10 repetitions (only data for samples having fungi present were analyzed. Standard deviations are in parentheses. ND, not detected; SM, spread molds.

Simplate studies for total counts and coliforms have shown that Simplate is suitable for estimating populations of mesophilic aerobic microorganisms (1, 10, 14), coliform bacteria, and *Escherichia coli* (13) in a wide range of food. However, for yeast and mold counts, Simplate was less highly correlated than other methods (r^2 , 0.88 to 0.90), such as PDA, DRBC, and Iso Grid (11).

Most studies on comparison of techniques to enumerate fungi have not discussed each food in detail. Depending on the food, one culture medium or technique may be more suitable than the other. Also, the mycobiota present in food has to be considered. Therefore, this work was carried out to compare the efficacy of three different culture media (DRBC, DG18, and PDA supplemented with two antibiotics), commonly used to enumerate fungi in food, versus the Simplate and Petrifilm techniques for molds and yeasts.

MATERIAL AND METHODS

Samples. Food samples were purchased from local supermarkets, and others were acquired from different food industries in Brazil. The following foods were analyzed: corn meal, wheat flour, cassava flour, bread crumbs, whole meal, sliced bread, ground peanuts, mozzarella cheese, grated parmesan cheese, cheese rolls, orange juice, pineapple pulp, pineapple cake, and mushroom in conserve.

Approximately 2 kg of each food sample was purchased or received. These 2 kg were divided into 10 subsamples, and from each subsample an aliquot of 25 g was weighed and analyzed separately. A total of 10 analyses were performed for each food.

Culture media. The culture media and techniques evaluated were (i) PDA (Difco Laboratories, Detroit, Mich.) plus antibiotics, i.e., chloramphenicol (100 mg liter⁻¹) and chlortetracycline (100 mg liter⁻¹); (ii) DG18 (Oxoid, Basingstoke, UK) (5); (iii) DRBC (Difco) (7); (iv) Petrifilm for molds and yeasts (3M Company, Minneapolis, Minn.); and (v) SYM (BioControl Systems, Inc., Bellevue, Wash.).

Subsample preparation, plating, and enumeration. Twenty-five-gram samples of food were homogenized with 225 ml of

0.1% peptone water using a stomacher 400 (Seward, London, UK). Aliquots of 1.0 ml were divided onto media on four petri dishes, or 0.1 ml from several dilutions was spread onto different culture media. One-milliliter aliquots were inoculated onto Petrifilm for yeasts and molds. The petri dishes and Petrifilm were incubated upright at 25°C for 5 days, after which mold and yeast colonies were counted (8).

The vial containing SYM test medium was hydrated with 100 ml of sterile water. This medium was divided into 10 tubes containing 9.0 ml of medium; the remaining medium was not used. A 1.0-ml aliquot of the subsample dilution was added to 9.0 ml of reconstituted SYM medium. The inoculated medium was then dispensed into a Simplate and incubated at 30° for 48 h. Another 24-h incubation at 30°C was also evaluated. Simplate wells fluorescing under UV light at a 365-nm wavelength (Chromato-vue Ultra Violet Products, Inc., San Gabriel, Calif.) were counted, and the most probable number of yeasts and molds in the original sample was determined using a table provided by the manufacturer.

Statistical analysis. Colony counts were first converted to \log_{10} counts to match the underlying assumption of normal distribution. Analysis of variance and Tukey's honestly significant difference test were used to compare the enumeration media, and standard regression techniques were used to calculate correlation coefficients, slopes, and intercepts. Coefficient of variation was calculated by dividing the repeatability standard deviation by the mean count and expressing it as a percentage. Only data for samples having countable fungi were analyzed.

RESULTS AND DISCUSSION

Mold and yeast counting from corn meal, wheat flour, cassava flour, bread crumbs, whole meal, sliced bread, ground peanuts, mozzarella cheese, grated parmesan cheese, cheese rolls, orange juice, pineapple pulp, pineapple cake, and mushroom in conserve are presented in Table 1. Spreading of mucoraceous molds (*Rhizopus* spp. and *Mucor* spp.) occurred in cassava flour, bread crumbs, and whole meal samples, which affected PDA, Simplate, and Petrifilm counting. This was not observed on DG18 and

DRBC plates. With the Simplate technique, the reading was carried out at 48 and 72 h, and in some foods a great difference was noted. At 48 h the fluorescence was doubtful and not reliable; a reincubation for 24 h was necessary. Spangenberg and Ingham (11) noticed the same problem. Simplate wells after 48 h of incubation at 30°C were weak and difficult to detect, so incubation was continued for another 24 h. Even with this additional incubation, results were sometimes equivocal.

Corn meal. The total counts on PDA, DG18, and DRBC were similar in this product. DG18 showed slightly higher counts than DRBC and PDA. Fungal overgrowth was not noticed in any of these culture media. Petrifilm had the lowest counts and Simplate the highest. Some wells on Simplate presented fungal growth, without presenting fluorescence; in this case, the wells were considered positive.

Wheat flour. DG18 had higher counting than PDA and DRBC, although these were similar. Simplate did not show fluorescence after 48 h, and even after 72 h this fluorescence was not well defined. Considering this as positive, the counts were higher than culture media. Petrifilm also showed a higher counting than culture media.

Cassava flour. *Mucor* and *Rhizopus* were present in cassava flour samples. PDA, Petrifilm, and Simplate plates were affected. Counting in Simplate was not possible because the plates were all covered after 48 h. The use of dichloran on DRBC and DG18 may reduce the overgrowth of these molds. Because it is difficult to predict if these molds will occur, the use of DG18 for food with an a_w below 0.95 is recommended (2, 5, 9). Taniwaki et al. (12) found that these species were very common in flour samples from tropical regions.

Bread crumbs. *Mucor* and *Rhizopus* also appeared in bread crumbs. However, the counting on PDA and Petrifilm was possible at higher dilution. On Simplate, the counting was carried out because the plates were not totally covered with these species. However, this technique gave lower results.

Whole meal. The counts were similar on PDA, DRBC, and DG18. *Mucor* and *Rhizopus* also appeared in this food. Similar to bread crumbs, at higher dilution, the counts were possible, because these species did not appear. On Simplate, these species appeared and spread over all plates, making the counting difficult. On Petrifilm, the counting was underestimated for the same reason.

Sliced bread. Fungal counts on PDA, DG18, and DRBC were similar. There was no growth of *Mucor* spp. and *Rhizopus* spp. Species of *Eurotium* predominated on all culture media. Petrifilm and Simplate counts were very similar to culture media. At 48 h there was no growth on Simplate. This may be because *Eurotium* species grow slowly and need longer periods of incubation.

Ground peanuts. There was overgrowth of *Mucor* and *Rhizopus* species on PDA, Petrifilm, and Simplate. However, at higher dilutions these species did not appear and

the counting could be carried out. On DRBC and DG18, growth of these species was restricted. Similar results were shown in PDA, DG18, DRBC, Petrifilm, and Simplate for this product.

Mozzarella cheese. Yeasts predominated in this product. The countings on PDA, DG18, and DRBC were similar. Petrifilm performed as well as all culture media. Yet on Simplate, the fluorescence was very unclear and doubtful.

Parmesan cheese. A great predominance of yeasts was noted. Fungal counts were similar in PDA, DG18, and DRBC. Petrifilm presented slightly lower counting than culture media, but yeast colonies were easily distinguished. Counting on Simplate was very difficult because there was no fluorescence at all and few wells presented fungal growth without fluorescence.

Cheese rolls. There was a predominance of yeast species. Counts on DRBC were slightly higher than on PDA and DG18. Counts on Petrifilm were similar to DRBC. On Simplate, it was very difficult to distinguish if there was fluorescence on the wells or not and the counting was underestimated.

Orange juice. Yeasts predominated in this product. Fungal counts were similar in all culture media and Petrifilm. Yeast growth was very visible on Petrifilm. On Simplate, the count was much lower.

Pineapple pulp. This product presented only yeasts. The counts were similar on PDA, DRBC, and DG18. On Petrifilm, the visibility and reading were clear, although the counting was slightly lower than culture media. On Simplate the counting was lower than the other techniques and the reading was possible only at 48 h because at 72 h all the wells were positive.

Pineapple cake. DG18 showed the highest counting in this product. PDA and DRBC had similar counts and Petrifilm was also similar. At 48 h on Simplate, the wells were all negative and the counting was carried out only at 72 h. Simplate had the lowest counting.

Mushroom in conserve. Yeasts predominated in this food. The counts were similar on three media with PDA, DG18, and DRBC. Petrifilm showed slightly higher counts than culture media. With Simplate, at 72 h the counts were similar to culture media but not at 48 h.

Considering DRBC and DG18 as standard media, Figures 1 and 2 show the correlation coefficients, slopes, and intercepts derived from comparing each culture media (DRBC, DG18, and PDA) and each technique (Petrifilm and Simplate) with each other from the composite of 14 foods.

DRBC versus PDA versus DG18. Correlation coefficients of DRBC versus PDA and DG18 for recovering total mold and yeast counts from the composite of 14 foods were 0.9903 and 0.9781, respectively. Slope and intercept values were, respectively, 1.0022 and 0.0327 for DRBC versus PDA and 0.9905 and 0.102 for DRBC versus DG18.

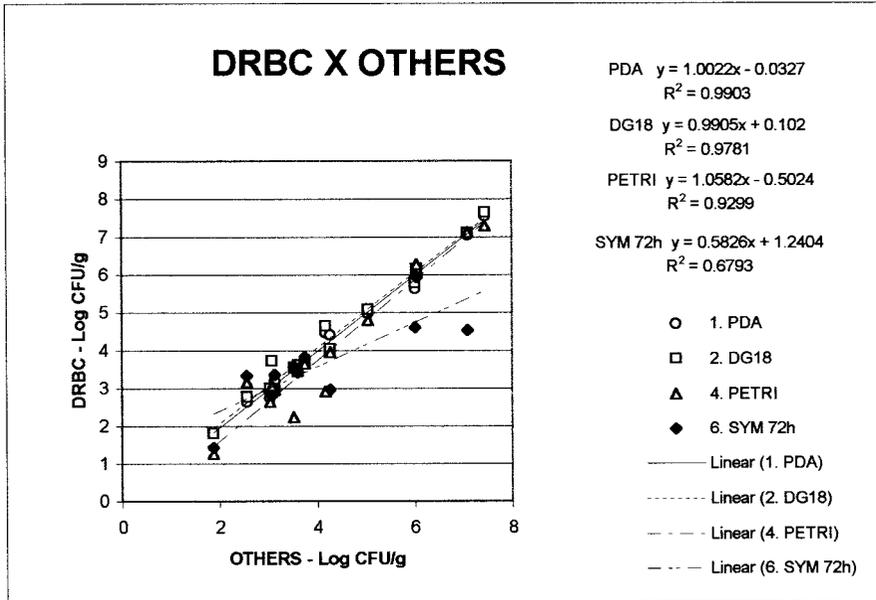


FIGURE 1. Relationship between DRBC log plate count CFU and log PDA, DG18, Petrifilm, and Simplate for the determination of molds and yeasts (linear regression).

For DG18 versus PDA, correlation coefficient, slope, and intercept were, respectively, 0.9797, 0.9953, and 0.0638. These results demonstrate that total counts of molds and yeasts on these three media, in general, were equivalent. Situations where a significant difference was observed among the results were restricted to only two foods, PDA versus DG18 orange juice analysis and DRBC versus DG18 whole meal and orange juice analysis. This finding confirms that for food with high a_w without the presence of spreading molds, DRBC and PDA are recommended. For food with a_w below 0.95, where xerophilic fungi are more common, DG18 is preferred (5, 8, 9).

Petrifilm versus DRBC versus DG18 versus PDA.

The correlation coefficient of Petrifilm versus DRBC for recovering total yeasts and molds from composites of 14 foods was 0.9299. Slope and intercept values were, respectively, 1.0582 and 0.5024. In most cases where a significant difference was observed, the Petrifilm underestimated the

count of molds and yeasts. These data demonstrate that although the correlation coefficient of Petrifilm versus DRBC, Petrifilm versus DG18, and Petrifilm versus PDA are still considered acceptable, the counts obtained with Petrifilm did not present such a good equivalence as that observed among DRBC, PDA, and DG18. Spangenberg and Ingham (11) found much higher correlation in part-skim mozzarella cheese in PDA, DRBC, Iso-Grid, and Petrifilm methods ($r^2 \geq 0.96$), and these methods were practically equivalent (regression equation slope, 0.91 to 1.06; y intercept, -0.13 to $0.51 \log_{10}$ unit). Mold and yeast counts in dairy products and acid foods, such as yogurt, relishes, sour cream, fruit juices, salad dressings, and sauces, presented the following correlation coefficients of Petrifilm versus pour-acidified PDA, surface-acidified PDA, pour plate count agar supplemented with chloramphenicol, and surface plate count agar supplemented with chloramphenicol, respectively: 0.993, 0.993, 0.994, and 0.995 (3). It seems that

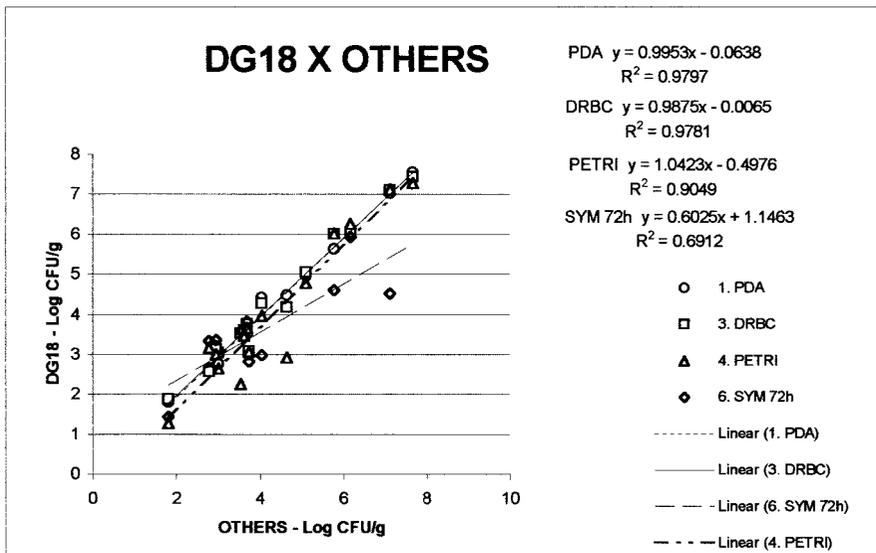


FIGURE 2. Relationship between D18 log plate count CFU and log PDA, DRBC, Petrifilm, and Simplate for the determination of molds and yeasts (linear regression).

for food with high yeast counts, without spreading molds and without xerophilic species, Petrifilm has good correlation with culture media. However, more studies are necessary for food with a_w below 0.95, where mold growth predominates and an improvement of the media inside the Petrifilm would be necessary.

Simplate. Correlation coefficients of Simplate versus DRBC, DG18, PDA, and Petrifilm for recovering total yeasts and molds from a composite of 11 foods (only data for samples having countable fungi were analyzed) were, respectively, 0.6793, 0.6912, 0.6146, and 0.6159. These data demonstrate that there was no equivalence among the counts obtained by Simplate and other culture media, with a significant difference being observed in the analysis of most products considered in the comparison (Figs. 1 and 2). In most of these cases, it was also observed that Simplate underestimated the count of molds and yeasts. The Simplate method was the least highly correlated method (r^2 , 0.88 to 0.90) compared with PDA, DRBC, and Iso-Grid for part-skim mozzarella cheese examination (11). Although Simplate techniques have been shown as suitable for estimating mesophilic aerobic counts, coliform bacteria, and *E. coli*, Simplate for yeast and mold counts needs improvement. According to the manufacturer's catalogue (Bio-Control Systems, Inc.), Simplate should not be used for raw flour products or for unprocessed fruits and vegetables because endogenous activity may be present in these products. Yet it can be used for fruit juices and juice concentrates, salad dressings, processed ingredients, processed flour products, sweetener products, dairy products, meat and poultry products, and processed fruits and vegetables. This makes Simplate a very limited technique, since raw flour products and unprocessed fruits and vegetables present major problems with fungi.

The food industry still needs a rapid and reliable technique to enumerate yeasts and molds in food. Currently, the alternative of replacing culture media is restricted to some foods.

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