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Evaluation of a Culture Film (Petrifilm™ YM) Method for Enumerating Yeasts and Molds in Selected Dairy and High-Acid Foods

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ABSTRACT

The Petrifilm™ Yeast and Mold (YM) plate was compared to acidified potato dextrose agar (APDA) and chloramphenicol-supplemented plate count agar (CPCA) using pour- and surface-plating techniques for its ability to recover yeasts and molds from hard and soft cheeses, cottage cheese, yogurt, sour cream, fruit juice, salad dressing, relishes, and tomato-based sauces. Correlation coefficients of Petrifilm™ YM plates versus pour-APDA, surface-APDA, pour-CPCA, and surface-CPCA for recovering total yeasts and molds from a composite of the eight test foods were, respectively, 0.993, 0.993, 0.994, and 0.995. Slope and intercept values for populations detected using Petrifilm™ YM plates versus traditional systems ranged, respectively, from 0.984 to 1.008 and -0.051 to 0.149. The coefficient of variation for total yeast and mold populations recovered on Petrifilm™ YM plates was 1.0% compared to 1.2 to 1.7% for traditional enumeration systems. Regardless of the enumeration system employed or the type of fungal cell, i.e., yeast or mold, being enumerated, significantly ($P \leq 0.05$) higher populations were generally detected after 5 d compared to 3 d of incubation. After 5 d of incubation, in no case were yeast or total yeast and mold populations detected in the eight food products using Petrifilm™ YM plates significantly lower than respective populations detected using traditional pour- and surface-plating techniques and media. When Petrifilm™ YM plates were used, significantly higher total yeast and mold populations were detected in 3, 1, and 1 out of eight food products compared to using, respectively, pour-APDA, surface-APDA, and surface-CPCA enumeration systems. The Petrifilm™ YM plate offers an acceptable alternative to traditional methods for enumerating yeasts and molds in the dairy and high-acid products evaluated in this study.

Yeasts and molds are distributed widely in decaying plant materials, soil, and air. Their presence on unprocessed plant and animal foodstuffs is almost assured by harvesting, handling, distribution, and storage practices used in the food industry, and inadequate preservation of these foodstuffs can result in mycological spoilage.

Detection and enumeration of yeasts and molds in foods is an integral part of any good quality assurance program and can reflect the effectiveness of sanitation practices, processing schemes, and distribution conditions. An ideal medium for enumerating yeasts and molds should suppress bacterial growth, be nutritionally adequate to support the growth of fastidious fungi, retard radial growth of colonies, and promote growth of yeasts and molds of relevance to the contamination or spoilage of the food being examined but not prohibit growth of irrelevant fungi (*14*).

Advances in methods for enumerating yeasts and molds in foods have lagged behind those made for enumerating bacteria. Traditionally, acidified potato dextrose agar (APDA) has been used to enumerate yeasts and molds in foods, but this medium does not provide an exceptionally good nutrient source, may not eliminate colony development by some bacteria, may inhibit resuscitation of injured cells, and may cause precipitation of food particles due to its low pH (*1,2,7,8,9,13*). Antibiotic-supplemented media are generally preferred to acidified media (*4,12*).

A relatively recent procedure for enumerating viable yeasts and molds in foods is the hydrophobic grid-membrane system (*3*). A more recent innovation in food mycology methodology has been the development of Petrifilm™ Yeast and Mold plates. The objective of this study was to compare Petrifilm™ YM plates to APDA and chloramphenicol-supplemented plate count agar (CPCA) for enumerating yeasts and molds in selected dairy products, fruit juices, salad dressings, relishes, and tomato-based sauces.

MATERIALS AND METHODS

Food products

Thirty lots each of eight food products (Table 1) were analyzed. None of the products contained added preservatives. Upon purchasing from local groceries, lots of some products were maintained at 25°C for periods up to 4 months to encourage growth of natural microbial contaminants. Relishes and sauces were the only commercially sterilized products examined. The majority of these products did not show evidence of microbial spoilage when purchased and were therefore inoculated with

TABLE 1. *Food products analyzed for yeast and mold populations.*

Product[1]	Product description
Hard and soft cheese	Cheddar, Swiss, Provolone, Mozzarella, Muenster, Neufchatel, Brie, blue, cream, and processed.
Cottage cheese	Large curd, small curd; regular and low fat
Yogurt	Plain, fruit, and fruit/nut
Sour cream	Plain, light, and with chives
Fruit juice	Refrigerated (not pasteurized)
Salad dressings	Refrigerated (not pasteurized), containing oil, vinegar, cheeses, buttermilk, sour cream, pickled vegetables, vegetable sauce/puree, honey, and/or spices
Relishes	Fermented and nonfermented vegetables and fruits, vinegar, sugar, and/or spices
Sauces	Tomato-based, including barbeque sauce and ketchup, and containing vinegar, vegetables, sugar, and/or spices

[1]Each product type consists of 30 lots. Duplicate samples from each lot were analyzed.

small amounts (<0.2%, v/v) of mixtures of fruit juices, salad dressings, and fermented dairy products which had undergone spoilage. Incubation at 25°C assured substantial growth of microflora in relishes and sauces within 2 to 3 weeks.

Enumeration systems

Five systems were evaluated for enumerating yeast and mold populations in the eight food products. The Petrifilm™ Yeast and Mold (YM) Plate (3M Company, Medical Products Division, St. Paul, MN) is a sample-ready system for the enumeration of yeasts and molds. The plate consists of a coating of Sabouraud agar Modified (Difco, Detroit, MI) supplemented to contain 3% glucose on a base film which is overlaid with a second film coated with a cold-water-soluble gelling agent. During incubation, chlortetracycline and chloramphenicol diffuse from the adhesive layer into the nutrient media to inhibit bacterial growth. The dimensions of each Petrifilm™ YM plate are 7.5 x 9.5 cm.

The other four systems consisted of using potato dextrose agar acidified to pH 3.5 with 10% tartaric acid (APDA) and plate count agar supplemented with chloramphenicol (100 μg/ml) (CPCA, pH 6.8) to which appropriately diluted samples were applied using pour- and surface-plating techniques.

Sampling procedure

Samples [10 g (ml)] from each lot were analyzed in duplicate. Each sample was combined with 90 ml of Butterfield's phosphate buffer (pH 7.0) and pummeled in a Colworth Stomacher for 30 sec. Samples were then serially diluted before depositing 1.0-ml aliquots in the center of Petrifilm™ YM plates. The cover film was lifted to facilitate application of the sample and then rolled back into place before samples were uniformly spread on the surface of each plate using a specially designed template. Likewise, 1.0 ml of serially diluted sample was deposited in Petri dishes (90 cm diam) to which ca. 18 ml of molten (47°C) APDA or CPCA were added; serially diluted 0.1-ml samples were deposited on the surfaces of APDA and CPCA plates which had been poured and "dried" at 25°C for 1 to 3 d. Samples were distributed in molten APDA and CPCA by gently moving plates in a circular motion around a ca. 6-cm radius, whereas distribution of samples on surfaces of solidified APDA and CPCA was achieved using sterile bent glass rods.

Incubation and enumeration procedures

Inoculated Petrifilm™ YM plates and traditional agar media plates were incubated in an upright position at 25°C. Colonies of yeasts and molds were each separately enumerated after 3 and 5 d. The total populations of yeasts and molds detected after 3 and 5 d were then calculated. Plates containing 15 to 150 colonies were selected for counting (*10*), although occasionally plates containing numbers of colonies outside this range were included. Cells from randomly picked presumptive yeast colonies were examined microscopically to confirm the absence of bacterial colonies.

Statistical analysis

Colony 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 Test were used to compare the enumeration methods in this study. Paired T-tests were used to compare day 3 to day 5 counts. 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 as a percentage.

RESULTS AND DISCUSSION

The vast majority of yeast colonies formed on Petrifilm™ YM plates were characterized by a blue coloration which was more distinct after 5 d of incubation compared to 3 d. Occasional white or cream colonies were also produced by yeasts on Petrifilm™ YM plates. Molds produced variously colored colonies but did not elicit the blue pigmentation characteristic of most yeasts. Yeast colonies were more confined than mold colonies on all enumeration systems evaluated. Microscopic examination of colonies was occasionally necessary to differentiate colonies of yeast from those of mold origin.

Populations of yeasts and molds detected in the eight food products using five enumeration systems are listed in Table 2. Molds were not detected in sour cream or salad dressing. This is not to say that viable mold propagules were not present in these products but rather that mold populations were not high enough to be detected in diluted samples which resulted in the development of yeast colonies in the range of 15 to 150 per plate. Values listed for yeasts plus molds for sour cream and salad dressing, then, actually represent populations of yeasts. Because results of analysis of foods for mycological quality are most often reported in terms of total yeasts and molds and because it was desirable to compare results across all eight food products, we chose to include values for yeasts as well as for yeasts plus molds for all food products listed in Table 2.

Correlation coefficients, slopes, and intercepts derived from comparing the five enumeration systems to each other

TABLE 2. *Populations of yeasts and molds detected in eight food products using Petrifilm™ YM plates, acidified potato dextrose agar (APDA) and chloramphenicol-supplemented plate count agar (CPCA).*[1]

Product	Micro-organism	Incu-bation time (d)	Population recovered (log_{10} CFU/g[ml]) and standard deviation[2]									
			Petrifilm™ YM plate		APDA pour		APDA surface		CPCA pour		CPCA surface	
Hard/soft cheese	Yeast	3	6.59 ab	(1.54)	6.54 b	(1.51)	6.60 ab	(1.52)	6.55 b	(1.48)	6.67 a	(1.52)
		5	*6.66 a	(1.55)	*6.58 b	(1.54)	*6.61 b	(1.53)	*6.62 ab	(1.52)	6.67 a	(1.53)
	Mold	3	5.29 b	(1.54)	5.35 ab	(1.55)	5.45 a	(1.51)	5.38 ab	(1.57)	5.45 a	(1.48)
		5	*5.37 c	(1.52)	*5.38 bc	(1.52)	*5.51 ab	(1.48)	*5.41 abc	(1.56)	*5.51 a	(1.47)
	Yeast + mold	3	6.68 b	(1.49)	6.65 b	(1.46)	6.70 b	(1.47)	6.67 b	(1.42)	6.78 a	(1.46)
		5	*6.76 ab	(1.49)	*6.69 c	(1.47)	*6.72 bc	(1.48)	*6.74 abc	(1.45)	*6.79 a	(1.46)
Cottage cheese	Yeast	3	5.12 b	(1.27)	5.53 b	(1.22)	5.62 a	(1.23)	5.50 b	(1.21)	5.66 a	(1.21)
		5	*5.59 abc	(1.24)	*5.70 bc	(1.21)	*5.64 ab	(1.22)	*5.54 c	(1.22)	*5.66 a	(1.21)
	Mold	3	5.16	(0.73)	5.15	(0.69)	5.27	(0.71)	5.19	(0.73)	5.22	(0.78)
		5	5.21	(0.70)	5.13	(0.73)	*5.28	(0.65)	5.18	(0.73)	5.24	(0.75)
	Yeast + mold	3	5.67 abc	(1.14)	5.65 c	(1.11)	5.76 ab	(1.13)	5.67 bc	(1.09)	5.78 a	(1.12)
		5	*5.74 abc	(1.11)	*5.67 c	(1.11)	*5.77 ab	(1.12)	5.69 bc	(1.10	*5.80 a	(1.12)
Yogurt	Yeast	3	5.76	(1.25)	5.75	(1.29)	5.77	(1.26)	5.79	(1.26)	5.79	(1.25)
		5	*5.77	(1.24)	*5.77	(1.27)	*5.77	(1.25)	5.81	(1.24)	*5.80	(1.25)
	Mold	3	5.19	(0.93)	5.28	(0.95)	5.30	(0.91)	5.23	(0.95)	5.28	(0.93)
		5	5.20	(0.92)	5.28	(0.95)	5.34	(0.90)	5.24	(0.96)	5.28	(0.93)
	Yeast	3	5.90	(1.21)	5.89	(1.25)	5.92	(1.21)	5.90	(1.24)	5.92	(1.21)
	+ mold	5	*5.91	(1.20)	5.90	(1.23)	5.92	(1.21)	5.92	(1.22)	*5.93	(1.21)
Sour cream	Yeast	3	7.84 a	(0.24)	7.83 ab	(0.30)	7.80 ab	(0.32)	7.82 ab	(0.32)	7.77 b	(0.28)
		5	7.84 a	(0.24)	7.83 ab	(0.30)	7.80 ab	(0.32)	7.82 ab	(0.32)	7.77 b	(0.28)
	Yeast + mold	3	7.84 a	(0.24)	7.83 ab	(0.30)	7.80 ab	(0.32)	7.82 ab	(0.32)	7.77 b	(0.28)
		5	7.84 a	(0.24)	7.83 ab	(0.30)	7.80 ab	(0.32)	7.82 ab	(0.32)	7.77 b	(0.28)
Fruit juice	Yeast	3	6.16	(1.12)	6.12	(1.22)	6.21	(1.10)	6.12	(1.11)	6.19	(1.06)
		5	6.19	(1.12)	*6.11	(1.22)	*6.21	(1.10)	6.14	(1.11)	6.20	(1.05)
	Mold	3	4.21	(0.93)	4.29	(0.92)	4.31	(0.91)	4.30	(0.99)	4.23	(1.01)
		5	4.22 b	(0.95)	*4.37 ab	(0.92)	*4.39 a	(0.95)	4.33 ab	(0.99)	4.25 ab	(0.98)
	Yeast + mold	3	5.60 ab	(1.41)	5.59 ab	(1.44)	5.64 a	(1.40)	5.56 b	(1.42)	5.60 ab	(1.44)
		5	5.61	(1.43)	*5.61	(1.42)	*5.65	(1.40)	5.58	(1.42)	*5.62	(1.41)
Salad dressing	Yeast	3	5.18	(1.47)	5.18	(1.46)	5.14	(1.44)	5.21	(1.43)	5.19	(1.41)
		5	*5.21 ab	(1.46)	*5.19 ab	(1.46)	5.15 b	(1.43)	*5.23 a	(1.43)	*5.20 ab	(1.41)
	Yeast + mold	3	5.18	(1.47)	5.18	(1.46)	5.14	(1.44)	5.21	(1.43)	5.19	(1.41)
		5	*5.21 ab	(1.46)	*5.19 ab	(1.46)	5.15 b	(1.43)	*5.23 a	(1.43)	*5.20 ab	(1.41)
Relish	Yeast	3	6.11 a	(0.74)	6.01 b	(0.80)	6.06 ab	(0.77)	6.09 ab	(0.73)	6.10 a	(0.72)
		5	*6.14 a	(0.73)	*6.02 b	(0.79)	6.07 ab	(0.77)	*6.11 a	(0.76)	*6.11 a	(0.72)

	Mold	3	4.36 ab	(0.42)	4.27 b	(0.45)	4.56 a	(0.48)	4.56 a	(0.37)	4.42 ab	(0.48)
		5	4.46 ab	(0.37)	4.30 b	(0.46)	4.59 a	(0.45)	4.59 a	(0.35)	*4.47 ab	(0.51)
	Yeast + mold	3	6.11 a	(0.74)	6.01 b	(0.80)	6.08 ab	(0.77)	6.10 a	(0.76)	6.10 a	(0.72)
		5	*6.15 a	(0.72)	*6.02 b	(0.79)	6.08 ab	(0.76)	*6.12 a	(0.75)	*6.12 a	(0.72)
Sauces	Yeast	3	5.57 a	(0.68)	5.40 c	(0.74)	5.49 b	(0.70)	5.53 ab	(0.72)	5.57 a	(0.70)
		5	*5.59 a	(0.68)	*5.41 c	(0.74)	*5.52 b	(0.70)	*5.54 ab	(0.72)	5.57 a	(0.70)
	Mold	3	3.86 b	(0.90)	4.33 a	(0.88)	4.36 a	(0.91)	4.35 a	(0.91)	4.38 a	(0.90)
		5	*4.26	(0.95)	4.33	(0.88)	4.40	(0.91)	*4.38	(0.91)	4.38	(0.90)
	Yeast + mold	3	5.58 ab	(0.68)	5.47 c	(0.74)	5.54 b	(0.70)	5.58 ab	(0.73)	5.62 a	(0.71)
		5	*5.63 a	(0.69)	*5.48 c	(0.73)	*5.55 b	(0.70)	*5.60 ab	(0.72)	5.62 a	(0.72)

[1]Thirty lots of each food product were analyzed in duplicate. Values listed within each product represent means of data from all lots of that product.

[2]Values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$). Values at 5 d for each type of microorganism within each product which are marked by an asterisk are significantly higher ($P \leq 0.05$) than values at 3 d for the same microorganism and product. Values in parentheses indicate standard deviations.

are presented in Table 3. The overall performance of Petrifilm™ YM plates was excellent when compared to traditional enumeration systems. Correlation coefficients of Petrifilm™ YM plates versus pour-APDA, surface-APDA, pour-CPCA, and surface-CPCA for recovering total yeasts and molds from composite of eight test foods were, respectively, 0.993, 0.993, 0.994, and 0.995. Slope and intercept values for populations detected using Petrifilm™ YM plates versus traditional media and plating techniques ranged, respectively, from 0.984 to 1.008 and -0.051 to 0.149.

The coefficients of variation of the five enumeration systems are shown in Table 4. Again, Petrifilm™ YM plates performed well. The coefficient of variation for total yeast and mold populations recovered on Petrifilm™ YM plates was 1.0% compared to a range of 1.2 to 1.7% for traditional enumeration systems. Except for yeast popula-

TABLE 3. *Statistical comparisons of yeast and mold populations detected in a composite of eight food products using Petrifilm™ YM plates, acidified potato dextrose agar (APDA), and chloramphenicol-supplemented plate count agar (CPCA).*[1]

			Petrifilm™ YM plate versus:				APDA surface versus:			CPCA surface versus:		
			APDA		CPCA			CPCA				
Measure of performance	Microorganism	Incubation time (d)	pour	surface	pour	surface	APDA pour	pour	surface	APDA pour	CPCA pour	APDA pour versus CPCA pour
Correlation coefficient	Yeast	3	0.992	0.992	0.993	0.993	0.993	0.992	0.996	0.993	0.994	0.995
		5	0.992	0.992	0.993	0.994	0.993	0.993	0.996	0.992	0.994	0.995
	Mold	3	0.964	0.996	0.959	0.968	0.983	0.982	0.982	0.979	0.979	0.981
		5	0.972	0.976	0.976	0.984	0.980	0.984	0.983	0.977	0.981	0.978
	Yeast + mold	3	0.994	0.992	0.993	0.994	0.995	0.993	0.995	0.994	0.995	0.996
		5	0.993	0.993	0.994	0.995	0.995	0.994	0.996	0.994	0.996	0.996
Slope	Yeast	3	0.979	1.000	1.008	1.021	0.973	0.999	1.014	0.954	0.982	1.023
		5	0.974	0.995	1.000	1.015	0.973	0.997	1.013	0.955	0.981	1.020
	Mold	3	0.976	0.990	0.956	0.971	0.981	0.964	0.961	0.994	0.977	0.965
		5	0.981	0.998	0.961	0.978	0.978	0.958	0.956	1.000	0.979	0.954
	Yeast + mold	3	0.984	0.998	1.000	1.006	0.980	0.995	1.003	0.972	0.990	1.013
		5	0.984	0.997	0.997	1.008	0.981	0.993	1.004	0.972	0.986	1.009
Intercept	Yeast	3	0.169	0.003	-0.036	-0.157	0.206	0.016	0.120	0.352	0.152	-0.170
		5	0.222	0.061	0.019	-0.089	0.199	0.006	-0.112	0.338	0.134	-0.167
	Mold	3	0.036	-0.135	0.063	-0.001	0.189	0.202	0.228	0.085	0.095	0.096
		5	0.065	-0.138	0.116	-0.023	0.218	0.268	0.276	0.055	0.107	0.176
	Yeast + mold	3	0.131	0.007	0.005	-0.067	0.157	0.041	-0.041	0.229	0.097	-0.105
		5	0.149	0.038	0.033	-0.051	0.145	0.038	-0.049	0.222	0.101	-0.094

[1]Comparisons of mean values are made from a composite of eight food products.

TABLE 4. *Coefficient of variation of Petrifilm™ YM plates, acidified potato dextrose agar (APDA), and chloramphenicol-supplemented plate count agar (CPCA) methods for enumerating yeasts and molds in a composite of eight food products.*

Time of incubation (d)	Microorganism	Coefficient of variation (%) Petrifilm™ YM plate	APDA pour	APDA surface	CPCA pour	CPCA surface
3	Yeast	1.5	1.7	1.5	1.5	1.8
	Mold	1.5	1.8	2.4	1.5	1.8
	Yeast + mold	0.9	1.0	1.8	1.4	1.6
5	Yeast	1.5	4.6	4.0	4.0	4.4
	Mold	4.5	4.7	5.1	4.2	5.0
	Yeast + mold	1.0	1.2	1.7	1.4	1.6

tions enumerated on Petrifilm™ YM plates, the coefficients of variation for yeast populations and mold populations detected using all test systems increased substantially after 5 d of incubation compared to 3 d. Examination of data by plotting $\log_{10}$ yeast plus mold populations after 5 d of incubation for Petrifilm™ YM plates versus populations detected on APDA and CPCA using surface and pour plating techniques (Fig. 1) further illustrated the performance of Petrifilm™ plates compared to traditional systems.

It was of great interest to determine if populations of yeasts and molds detected on Petrifilm™ YM plates after 3 d of incubation accurately reflected populations detected after 5 d. The procedure outlined by the U.S. Food and Drug Administration (*11*) prescribes counting colonies after 5 d of incubation and cautions against counting at 3 d since handling of plates could result in secondary growth from dislodged spores, making 5-d counts invalid. The American Public Health Association (*10*) suggests counting colonies after 3 d if excessive mold growth develops, and again after 5 d. Some fungal propagules simply will not form detectable colonies within 3 d when plated on media traditionally used for enumeration. The need for an incubation period longer than 3 d is again confirmed in the present study. Regardless of the enumeration system employed or the type of fungal cell (yeast or mold) being enumerated, significantly ($P \leq 0.05$) higher populations were generally detected after 5 d compared to 3 d of incubation (Table 1). Shown in Table 5 is information summarizing the number of times [out of eight (yeast, yeast plus mold) or six (mold)] populations detected in eight food products were significantly higher after 5 d of incubation compared to 3 d. Values are presented for each enumeration system. Clearly, the accuracy of enumeration is improved when the incubation time is extended from 3 to 5 d.

A comparison of Petrifilm™ YM plates to pour- and surface-plated APDA and CPCA with regard to suitability to recover yeasts and molds from food products is presented in summary form in Table 6. After 5 d of incubation, in no case were yeast or yeast plus mold populations detected in the eight food products using Petrifilm™ YM plates significantly lower than respective populations detected using traditional pour- and surface-plating techniques and media. When Petrifilm™ plates were used, significantly higher total yeast and mold populations were detected in 3, 1, and 1 out of eight food products compared to using, respectively, pour-APDA, surface-APDA, and surface-CPCA enumeration systems. Likewise, significantly higher populations of yeasts were detected on Petrifilm™ YM plates after 5 d of incubation. Conversely, recovery of molds on surface-plated APDA and CPCA was significantly better,

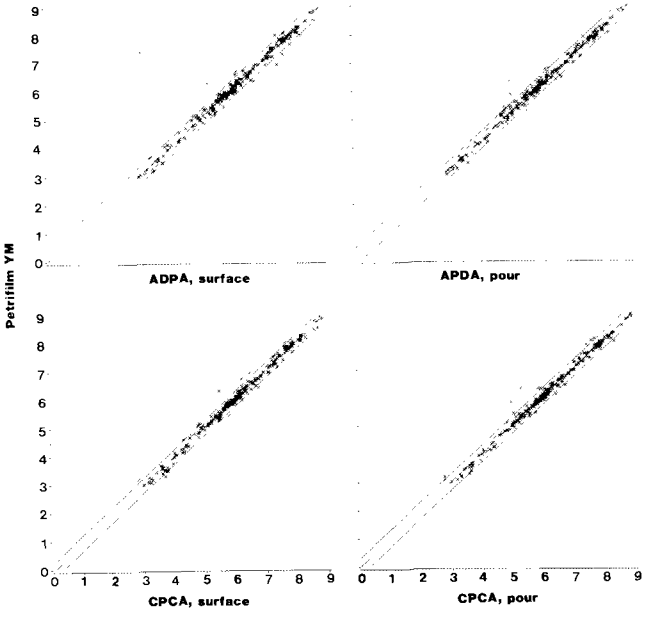

Figure 1. *Relationship of $\log_{10}$ yeast plus mold populations after 5 d of incubation determined by Petrifilm™ YM plates versus APDA (surface and pour) and CPCA (surface and pour) plating systems. Data represent a composite of 30 lots of eight food products and are indicated by least squares linear regression lines (solid lines) with 95% confidence limits (dashed lines).*

TABLE 5. *Comparison of yeast and mold populations detected using Petrifilm™ YM plates, acidified potato dextrose agar (APDA), and chloramphenicol-supplemented plate count agar (CPCA).*

Microorganism	Number of food products[1]	Enumeration system[2] Petrifilm™ YM plate	APDA pour	APDA surface	CPCA pour	CPCA surface
Yeast	8	6	7	5	5	4
Mold	6	2	2	3	2	2
Yeast + mold	8	6	6	4	4	6

[1]Yeast and yeast plus mold populations in all eight food products were determined. No molds were detected in sour cream and salad dressing.

[2]Values indicate the number of times [out of 8 (yeast, yeast plus mold) or 6 (mold)] populations were significnatly higher ($P < 0.05$) after 5 d of incubation compared to 3 d.

TABLE 6. *Comparison of Petrifilm™ YM plates to acidified potato dextrose agar (APDA) and chloramphenicol-supplemented plate count agar (CPCA) for enumerating yeast and mold populations.*

Time of incubation (d)	Microorganism	Number of food products[1]	Number of times populations detected on Petrifilm™ YM plates were significantly higher/lower[2] than on: APDA pour	APDA surface	CPCA pour	CPCA surface
3	Yeast	8	2/0	1/1	0/1	1/1
	Mold	6	0/1	0/2	0/1	0/2
	Yeast + mold	8	2/0	0/0	0/0	1/1
5	Yeast	8	3/0	2/0	0/0	1/0
	Mold	6	0/0	0/2	0/0	0/1
	Yeast + mold	8	3/0	1/0	0/0	1/0

[1]Yeast and yeast plus mold populations in all eight food products were determined. No molds were detected in sour cream and salad dressing.

[2]Values indicate the number of times [out of 8 (yeast, yeast plus mold) and 6 (mold)] a population detected on Petrifilm™ YM plates was significnatly ($P < 0.05$) higher/lower than the population detected on an alternate enumeration system.

respectively, for 2 and 1 of six food products after 5 d of incubation. This may be due to a more favorable oxygen tension associated with the surface-plating technique.

Although the results of this study demonstrate that Petrifilm™ YM plates are as good as or better than commonly used traditional methods for enumerating total yeast and mold populations in eight dairy and high-acid food products, it was also of interest to directly compare the performance of pour- and surface-plated APDA and CPCA. These comparisons are summarized in Table 7. Within each medium, the surface-plating technique was superior to the pour-plating technique. Considering one type of plating technique, CPCA clearly performed better than APDA. These results are not surprising. Exposure of stressed yeasts and molds to acid pH in recovery media can result in decreased populations (*2,13,14*). Likewise, exposure of stressed cells to elevated temperatures associated with pour plating can impose additional stress, perhaps resulting in death (*2,5,6*). The availability of oxygen to yeasts and molds is, of course, increased when using the surface-plating technique, thus enhancing colony development. In addition to the more favorable nutrient content in CPCA compared to APDA, the high pH (6.8) of CPCA is more favorable for resuscitating stressed cells.

Aside from its favorable performance as a system for enumerating yeasts and molds in foods, Petrifilm™ YM plates offer several inherent advantages over traditional systems. Among these are that no preparation time is required by the user, no tartaric acid or antibiotic addition is required, less space is required for storing and incubating, less time is required for plating samples, and secondary colony development is essentially eliminated. There is no need for an autoclave unless sterilization of Petrifilm™ YM plates is desired after analysis is completed nor is there a need for a waterbath to keep agar in a molten state. A disadvantage is that a few yeasts do not develop blue color, thus necessitating microscopic examination of colonies to facilitate differentiation from possible bacterial colonies. Occasionally, very small nonpigmented colonies are difficult to see, making the actual counting process potentially tedious to anyone not familiar with the technique. In addition, picking colonies of some fungal species from Petrifilm™ YM plates for the purpose of identification may be more difficult compared to traditional plating techniques. None of the systems evaluated was exceptionally effective at controlling colony development by *Rhizopus* and *Mucor*. Withstanding these limitations, the Petrifilm™ YM plate can be used very successfully to accurately enumerate yeasts and molds in the dairy and high-acid foods selected for evaluation in this study. Additional work is underway to determine the performance of Petrifilm™

TABLE 7. *Comparison of acidified potato dextrose agar (APDA) and chloramphenicol-supplemented plate count agar (CPCA) for enumerating yeasts and molds.*

Time of incubation (d)	Microorganism	Number of food products[1]	Number of times a population detected using a particular combination of plating technique and medium was significantly higher than that detected on an alternate plating technique and/or medium[2]: APDA (pour/surface)	CPCA (pour/surface)	Pour (APDA/CPCA)	Surface (APDA/CPCA)	APDA pour/ (CPCA surface)	CPCA pour/ APDA surface
3	Yeast	8	0/2	0/2	0/1	0/1	0/4	0/1
	Mold	6	0/1	0/0	0/1	0/0	0/0	0/0
	Yeast + mold	8	0/2	0/2	0/2	0/2	0/4	0/1
5	Yeast	8	0/1	0/1	0/2	0/2	0/4	1/1
	Mold	6	0/1	0/1	0/1	0/0	0/1	0/1
	Yeast + mold	8	0/2	0/1	0/2	0/2	0/4	1/0

[1]Yeast and yeast plus mold populations in all eight food products were determined. No molds were detected in sour cream and salad dressing.

[2]Values indicate number of times [out of 8 (yeast, yeast plus mold) and 6 (mold)] a population detected using a particular enumeration system was significantly ($P < 0.05$) higher than the population detected on an alternate system.

YM plates for enumerating yeasts and molds in a wide variety of other food products.

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