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Performance of mycological media in enumerating desiccated food spoilage yeasts: an interlaboratory study

L.R. Beuchat [a,*], E. Frändberg [b], T. Deak [a,c], S.M. Alzamora [d], J. Chen [a], S. Guerrero [d], A. López-Malo [e], I. Ohlsson [f], M. Olsen [b], J.M. Peinado [g], J. Schnurer [f], M.I. de Siloniz [g], J. Tornai-Lehoczki [h]

[a] *Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA*

[b] *Biology Division, National Food Administration, 751 26 Uppsala, Sweden*

[c] *Department of Microbiology and Biotechnology, Szent Istvan University, Somloi ut 14-16, 1118 Budapest, Hungary*

[d] *Pabellón de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina*

[e] *Departmento de Ingenieria Quimica y Alimentos, Universidad de la Americas-Puebla, Sta. Catarina Mártir, Puebla 72820, Mexico*

[f] *Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, 750 07 Uppsala, Sweden*

[g] *Departmento de Microbiologia, Facultad de Biologia, Universidad Completense de Madrid, 20840 Madrid, Spain*

[h] *National Collection of Agricultural and Industrial Microorganisms, Szent Istvan University, Somloi ut 14-16, 1118 Budapest, Hungary*

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Abstract

Dichloran 18% glycerol agar (DG18) was originally formulated to enumerate nonfastidious xerophilic moulds in foods containing rapidly growing *Eurotium* species. Some laboratories are now using DG18 as a general purpose medium for enumerating yeasts and moulds, although its performance in recovering yeasts from dry foods has not been evaluated. An interlaboratory study compared DG18 with dichloran rose bengal chloramphenicol agar (DRBC), plate count agar supplemented with chloramphenicol (PCAC), tryptone glucose yeast extract chloramphenicol agar (TGYC), acidified potato dextrose agar (APDA), and orange serum agar (OSA) for their suitability to enumerate 14 species of lyophilized yeasts. The coefficient of variation for among-laboratories repeatability within yeast was 1.39% and reproducibility of counts among laboratories was 7.1%. The order of performance of media for recovering yeasts was TGYC > PCAC = OSA > APDA > DRBC > DG18. A second study was done to determine the combined effects of storage time and temperature on viability of yeasts and suitability of media for recovery. Higher viability was retained at −18°C than at 5°C or 25°C for up to 42 weeks, although the difference in mean counts of yeasts stored at −18°C and 25°C was only 0.78 $\log_{10}$ cfu/ml of rehydrated suspension. TGYC was equal to PCAC and superior to the other four media in recovering yeasts stored at −18°C, 5°C, or 25°C for up to 42 weeks. Results from both the interlaboratory study and the storage study support the use of TGYC for

* Corresponding author. Tel.: +1-770-412-4740; fax: +1-770-229-3216.
E-mail address: lbeuchat@cfs.griffin.peachnet.edu (L.R. Beuchat).

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enumerating desiccated yeasts. DG18 is not recommended as a general purpose medium for recovering yeasts from a desiccated condition. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Mycological media; Yeast; TGYC

1. Introduction

Yeasts are susceptible to structural and metabolic injury when exposed to environmental stresses such as temperature extremes, chemicals, and desiccation (Beuchat, 1984; Deak and Beuchat, 1996). Injured cells may have increased sensitivity to otherwise innocuous environments imposed by enumeration media. Heat-stressed yeasts have increased sensitivity to acidic environments (Menegazzi and Ingledew, 1980; Nelson, 1972; Jarvis and Williams, 1987) but freeze-stressed yeasts appear to be less affected by low pH in recovery media (Beuchat and Nail, 1985). Enumeration of *Zygosaccharomyces rouxii* adapted to or stressed by reduced a_w is influenced by the a_w and composition of recovery media (Abdul-Raoulf et al., 1994; Hernandez and Beuchat, 1995). The type of solute used to achieve a given a_w in recovery media may influence resuscitation of osmotically stressed yeast cells (Beuchat and Hocking, 1990). To detect all viable yeast cells in foods with reduced a_w, conditions for repair in the recovery medium must be optimized.

Although acidified media are not recommended for enumerating yeasts and moulds in most foods (Beuchat and Cousin, 2000), some laboratories continue to use them. Enumeration media containing antibiotics to inhibit bacterial growth or various chemicals to control spreading of moulds have come into favour in recent years. Dichloran rose bengal chloramphenicol agar (DRBC), modified from a formula developed by King et al. (1979), which contained chlortetracycline, is a recommended general purpose medium for enumerating yeasts and moulds. However, photodegradation of rose bengal results in reactive oxygen species that inhibit *Saccharomyces cerevisiae* (Chilvers et al., 1999) and perhaps other yeasts. Dichloran 18% glycerol agar (DG18) was developed to enumerate nonfastidious xerophilic moulds in the presence of fast-growing *Eurotium* species and mucoraceous moulds (Hocking and Pitt, 1980). Some laboratories are using DG18 as a general purpose medium for enumerating yeasts and moulds, although the medium has not been evaluated for its performance in supporting recovery of food spoilage yeasts from foods with a_w less than that required for growth.

In another interlaboratory study (Deak et al., 2001), DRBC, DG18, tryptone glucose yeast extract chloramphenicol agar (TGYC), and plate count agar (PCA) supplemented with chloramphenicol (PCAC) were evaluated for their ability to support colony development by 14 food spoilage yeasts. Test cells were in an unstressed condition, i.e., in an early stationary growth phase. The study reported here was undertaken to evaluate these media, in addition to acidified potato dextrose agar (APDA) and orange serum agar (OSA), for their suitability to support colony development by the same 14 strains of yeasts that had been stressed by desiccation. Researchers from eight laboratories in six countries participated.

2. Materials and methods

2.1. Yeast strains

Fourteen yeast species representing 12 genera were used: *Brettanomyces anomalus* NRRL 1415, *Candida parapsilosis* ATCC 22019, *Cryptococcus albidus* DS-197, *Debaryomyces hansenii* NRRL 7268, *Hanseniaspora uvarum* CFSQE 77, *Issatchenkia orientalis* NRRL 7179, *Kluyveromyces marxianus* UCD 61293, *Pichia anomala* NRRL 3668, *P. membranifaciens* UCD 5722, *Rhodotorula mucilaginosa* CFSQE 63, *S. cerevisiae* KE 162, *Torulaspora delbrueckii* CFSQE 73, *Zygosaccharomyces bailii* NRRL 7256, and *Z. rouxii* ATCC 52519.

2.2. Procedure for lyophilizing yeasts

All yeasts were lyophilized at the National Food Administration, Uppsala, Sweden. Each strain was

inoculated onto agar slants and incubated for 4 days at 25 ± 1°C. All strains except *Z. rouxii* were cultivated on malt extract agar (Oxoid, Basingstoke, UK). *Z. rouxii* ATCC 52519 was cultivated on DG18 (Oxoid). Cultures (10 μl) were streaked on 200 ml of respective agars in screw capped 500-ml Duran bottles and incubated at 25 ± 1°C for 4 days. Cells from each bottle were harvested by flooding the surface of agar with 50 ml of sterile skimmed milk supplemented with inositol (5%) and gently agitating. Suspensions (0.5 ml) were distributed into sterile 2-ml glass vials and frozen at −35°C. Cell suspensions were freeze dried using a Christ freeze drier (Epsilon 1-12D, Osterode, Germany) according to the procedure described by Smith and Onions (1994). Vials were sealed with rubber bungs under vacuum and stored in the dark at 4 ± 1°C until dispatch via courier to collaborating laboratories.

2.3. Enumeration media

Six agar media were evaluated for their suitability to support colony development by lyophilized yeasts immediately after rehydration: DG18 (Oxoid, pH 6.5); DRBC base (Oxoid) supplemented with chloramphenicol (100 μg ml^{-1}) (DRBC, pH 5.6); PCA (Oxoid or Difco, Detroit, MI, USA) supplemented with chloramphenicol (100 μg ml^{-1}) (PCAC, pH 7.0); tryptone glucose yeast extract chloramphenicol agar (TGYC) (pH 6.0), which consists of 5.0 g of tryptone (Difco), 5.0 g of yeast extract (Difco), 100 g of anhydrous glucose, 100 mg of chloramphenicol, 15 g of agar, and 1 l of deionized water; PDA (Oxoid or Difco) supplemented with sterile 10% tartaric acid after autoclaving and cooling to 47–50°C to reduce to the pH to 3.5 (APDA); and OSA (Oxoid or Difco, pH 5.5). Media were prepared, poured into Petri dishes, and held 1–3 days before samples (0.1 ml) of rehydrated lyophilized yeasts were applied and surface spread. Collaborators were requested to store media in darkness prior to use.

2.4. Procedure for rehydrating, plating, and incubating yeasts

Collaborators were requested to store lyophilized yeasts at 4 ± 1°C in darkness and to conduct analysis to determine the performance of test media within 3 weeks of receipt of cultures from the National Food Administration. Collaborators were also requested to record the manufacturer's name, date of manufacture (or expiration date) of media, and date each medium was used in the study.

Each laboratory analyzed at least one vial of each lyophilized yeast. In a few cases, inappropriate dilution of samples required a second vial to be analyzed. One laboratory analyzed duplicate vials of all 14 yeasts, reporting data as two separate analyses. To the content of each vial, 1.0 ml of sterile 0.1% Bacto peptone (Difco) water was added. The lyophilized sample was vigorously shaken to suspend in peptone water, then transferred to a test tube containing 2 ml of sterile 0.1% peptone water; the use of 1 ml of 0.1% peptone to rinse the vial was repeated two more times. The suspension (ca. 5 ml) was held at 21 ± 2°C for 30 min before shaking, serially diluting in sterile 0.1% peptone water, and surface plating duplicated 0.1-ml samples on DG18, DRBC, PCAC, TGYC, APDA and OSA. Plates were incubated in darkness at 25°C for 5 days (±2 h) before counting colonies on plates supporting the development of 15–150 colonies. Observations on size, color, general appearance, and ease of counting colonies on enumeration media were recorded. All raw data were sent to the Center for Food Safety, University of Georgia for statistical analysis.

Additional studies were done at the University of Georgia to determine survival characteristics of lyophilized yeasts as affected by storage temperature and to determine if the performance of media in supporting colony development by yeasts differed as affected by the time and temperature of storage. Vials of 14 lyophilized yeasts received from the National Food Administration were stored at −18°C, 5°C, and 25°C for 10, 28 and 42 weeks. The initiation of this experiment was 2 weeks after yeasts were lyophilized. The same six media used by collaborating laboratories to enumerate yeasts shortly after lyophilization were also used to enumerate stored lyophilized yeasts.

2.5. Statistical analyses

Tabulated raw data were evaluated by analysis of variance using the general linear models procedure

of the SAS System (SAS Institute, Cary, NC, USA). Significant differences in mean values are reported at $\alpha = 0.05$.

3. Results and discussion

B. anomalus was the most fastidious among the yeasts included in this study. In a previous collaborative study (Deak et al., 2001), only two of seven laboratories reported recovery of stationary phase cells on all four enumeration media (DG18, DRBC, PCAC, and TGYC). Inconsistent results were again obtained in the present study. None of the eight laboratories reported counts for *B. anomalus* on all six enumeration media. However, the inability to obtain counts on a particular medium was not consistent among laboratories. Data from *B. anomalus* were, therefore, deleted from the analysis.

Two laboratories were unable to obtain OSA within a reasonable time after receiving the lyophilized cultures. Counts reported by these laboratories for test yeasts on the other five media were consistently 1–2 $\log_{10}$ cfu/ml lower than counts reported by the other six laboratories. The receipt of lyophilized cultures by these two laboratories was delayed, as was analysis after receipt. The potential for temperature abuse may have resulted in reduction in number of viable cells in the lyophilized cultures. In a study done to simulate changes in temperature during long transport of lyophilized mould cultures to a distant laboratory, Frändberg and Olsen (1999) observed that the decline in mean counts ranged from 0% to 50%, depending on the species. A similar phenomenon may have occurred in our study, with considerably greater reductions in numbers of viable yeast cells in cultures analyzed in the two laboratories reporting consistently lower values. For these reasons, data from these laboratories were not included in the analysis. Rather, data reflected the results reported by the other six laboratories.

Results of analysis of variance of data obtained from 13 yeasts on six enumeration media in six laboratories are shown in Table 1. Significant interactions occurred between these parameters. High values for yeasts reflect large differences in populations of various species and strains in lyophilized samples. Because of these significant interactions, data were analyzed within yeast and within laboratory. Table 2 shows mean populations of each of 13 yeasts recovered on six media by the six collaborating laboratories. R^2 values ranged from 0.880 for *Z. bailii* to 0.996 for *H. uvarum*. The among laboratories repeatability within yeast ranged from 0.89% for *R. mucilaginosa* to 2.40% for *D. hansenii*, with an overall repeatability of 1.39%. Among laboratories reproducibility was 7.1%. With the exceptions of counts for *H. uvarum* on DRBC and *Z. bailii* on DG18 and PCAC, TGYC was equal or significantly ($\alpha = 0.05$) better than the other five enumeration media in supporting the development of the highest number of colonies by rehydrated lyophilized yeasts. The order of performance was TGYC > PCAC = OSA > APDA > DRBC > DG18.

Table 1
Summary of analysis of variance of populations of 13 yeasts recovered on six media by six collaborating laboratories

Source	*df*	Sum of squares	Mean square	*F*-value	Pr > *F*
Replicates	1	0.0077	0.0077	1.57	0.2114
Laboratories	5	18.0786	3.6157	739.23	0.0001
Yeasts	12	246.7251	20.5604	4203.54	0.0001
Media	5	6.3108	1.2622	258.04	0.0001
Yeasts × media	60	12.5909	0.2098	42.90	0.0001
Lab × media	25	17.1975	0.6879	140.64	0.0001
Lab × yeasts	59	26.8018	0.4543	92.87	0.0001

Shown in Table 3 are mean populations of 13 yeasts recovered on six media by each of six collaborating laboratories. R^2 values were 0.983–0.999, while between laboratory repeatability ranged from 0.54% to 1.77%. Only in laboratories 3 and 6 were the performances of PCAC and TGYC, respectively, not equal or superior to all other test media in supporting colony development by the 13 yeasts.

Other researchers have demonstrated the high performance of TGY, a medium not containing chloramphenicol, for enumerating yeasts. Beuchat et al. (1998) showed that higher numbers of *Z. rouxii* were recovered from high sugar foods on TGY compared to DG18 or malt extract yeast extract 50% glucose agar (MY50G). Andrews et al. (1997) reported that DRBC recovered lower numbers of most of the five yeast species tested. In general, the growth of yeasts on DG18 was more variable and

Table 2
Populations of lyophilized yeasts recovered on six media by six collaborating laboratories

Yeast	R^2	CV (%)[a]	Population ($\log_{10}$ cfu/ml)[b]					
			DG18	DRBC	PCAC	TGYC	APDA	OSA
C. krusei	0.889	0.96	5.12c	5.14bc	5.18a	5.17ab	5.18ab	5.17ab
C. parapsilosis	0.972	0.99	5.31a	5.10c	5.22b	5.33a	5.31a	5.31a
Cry. albidus	0.963	1.45	5.30a	5.07b	5.33a	5.32a	5.27a	5.31a
D. hansenii	0.953	2.40	5.42a	5.10b	5.42a	5.51a	5.50a	5.47a
H. uvarum	0.996	0.98	4.41e	4.96a	4.77c	4.84b	4.71d	4.88b
K. marxianus	0.994	1.55	4.87c	4.60d	5.38a	5.38a	5.27b	5.36a
P. anomala	0.980	1.08	5.60a	5.58a	5.55a	5.58a	5.57a	5.59a
P. membranifaciens	0.967	0.96	5.16c	5.19bc	5.25a	5.24a	5.20bc	5.23ab
R. mucilaginosa	0.993	0.89	4.39e	4.74c	4.81b	4.86a	4.56d	4.82ab
S. cerevisiae	0.992	1.65	3.26e	3.50c	3.93a	3.90a	3.34d	3.85b
T. delbrueckii	0.917	1.33	5.45ab	5.41b	5.49a	5.48a	5.51a	5.47a
Z. bailii	0.880	2.01	4.40a	4.26b	4.41a	4.30b	4.13c	4.24b
Z. rouxii	0.983	1.18	5.04c	4.90d	5.19b	5.26a	4.93d	5.15b
Mean			4.90e	4.91d	5.07b	5.09a	4.96c	5.06b

[a]Coefficient of variation (repeatability) within yeast for six laboratories.
[b]Population detected in rehydrated yeast suspension. Mean values in the same row that are followed by the same letter are not significantly different ($\alpha = 0.05$).

weaker than on TGY. In a collaborative study comparing the performance of DG18, DRBC, MY50G, and oxytetracycline glucose yeast extract agar (OGY), Braendlin (1996) concluded that DG18 was superior for enumerating xerophilic yeasts in the presence of xerophilic moulds. The ability of collaborators to detect and count yeast colonies on DG18, DRBC, and OGY varied, however, depending on the sample. A yoghurt whey agar (pH 4.0) formulated and evaluated by Yamani (1993) proved to be comparable to APDA but inferior to PCA containing both chloramphenicol and chlortetracycline at 100 $\mu g\ ml^{-1}$ for enumerating yeasts in 22 food samples. Results of our study are in agreement with observations of these researchers showing that, overall, TGYC and PCAC perform better than media with reduced a_w or pH for enumerating food spoilage yeasts representing a wide range of physiological characteristics and tolerance to extrinsic stress factors.

A second study was done at the University of Georgia to determine the combined effects of storage

Table 3
Mean populations of 13 yeasts recovered on six media by six collaborating laboratories

Laboratory number	R^2	CV (%)[a]	Population ($\log_{10}$ cfu/ml)[b]					
			DG18	DRBC	PCAC	TGYC	APDA	OSA
1	0.994	1.42	5.18b	5.16b	5.23a	5.26a	5.05c	5.19b
2	0.990	1.53	5.12bc	5.08c	5.17a	5.15ab	5.02d	5.13bc
3	0.999	0.54	4.97c	5.03b	4.56c	5.05a	5.02b	5.05a
4	0.991	1.73	4.82c	4.39e	4.94a	4.96a	4.75d	4.88b
5	0.983	1.77	5.09a	5.00b	5.06a	5.11a	4.96b	5.08a
6	0.999	0.76	4.14e	4.70d	5.04a	5.00b	4.93c	5.05a
Mean			4.90e	4.91d	5.07b	5.09a	4.96c	5.06b

[a]Coefficient of variation (repeatability) within laboratory for 13 yeasts.
[b]Population detected in rehydrated suspension. Mean populations of all yeast recovered on each medium. Values in the same row that are followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 4
Summary of analysis of variance of populations of 13 yeasts recovered on six media after storing for 42 weeks at −18°C, 5°C, and 25°C[a]

Source	*df*	Sum of squares	Mean square	*F* value	Pr > *F*
Replicates	1	0.0073	0.0073	0.66	0.4186
Yeasts	12	192.9734	16.0811	1443.48	0.0001
Media	5	3.5083	0.7017	62.98	0.0001
Temperature	2	52.5240	26.2620	2357.34	0.0001
Yeasts × media	60	5.4781	0.0913	8.20	0.0001
Yeasts × temperature	24	22.3662	0.9319	83.65	0.0001
Media × temperature	10	0.3261	0.0326	2.93	0.0018
Yeast × media × temperature	120	1.1996	0.0099	0.90	0.7452

[a]Yeast species and enumeration media are listed in Table 2.

time and temperature on viability of 13 yeasts. The suitability of media for recovering yeasts was also evaluated. Data from 10- and 28-week storage studies (not shown) were similar to data for the 42-week study. Results of analysis of variance of data obtained from yeasts stored for 42 weeks at −18°C, 5°C, and 25°C are shown in Table 4. As with the interlaboratory study, significant interactions occurred between parameters, the highest values being for yeasts, again reflecting large differences in populations of various species in the lyophilized cultures.

Table 5 shows mean composite populations of each of the 13 yeasts stored for 42 weeks at −18°C, 5°C, and 25°C when plated on six enumeration media. Initial populations (Table 2) did not decrease substantially. R^2 values ranged from 0.808 for *P. anomala* to 0.997 for *P. membranifaciens*. The coefficient of variation ranged from 0.87% for *P. anomala* to 4.87% for *H. uvarum*. TGYC was equal to PCAC and superior to the other four media in recovering the highest number of yeasts. The overall order of performance of media was similar to that observed in the interlaboratory study using unstressed yeasts (Table 2). However, APDA supported the development of significantly lower numbers of colonies than did the other five media. Recovery on

Table 5
Populations of yeasts recovered on six media after storing for 42 weeks at −18°C, 5°C, and 25°C

Yeast	R^2	CV (%)[a]	Population ($\log_{10}$ cfu/ml)[b]					
			DG18	DRBC	PCAC	TGYC	APDA	OSA
C. krusei	0.916	1.40	4.98ab	4.97ab	5.03a	4.98ab	4.96ab	4.92b
C. parapsilosis	0.960	1.85	5.13ab	5.17a	5.04b	5.23a	5.13ab	5.22a
Cry. albidus	0.937	2.37	5.21a	5.15a	5.25a	5.27a	5.17a	5.24a
D. hansenii	0.960	2.22	5.34ab	5.28ab	5.30ab	5.39a	5.23b	5.28ab
H. uvarum	0.918	4.87	4.36bc	4.65a	4.58ab	4.56ab	4.12c	4.57ab
K. marxianus	0.955	1.32	5.47a	5.44ab	5.49a	5.48a	5.37b	5.43ab
P. anomala	0.808	0.87	5.84a	5.79a	5.82a	5.83a	5.80a	5.84a
P. membranifaciens	0.997	1.07	4.83bc	4.79c	4.89ab	4.87ab	4.72d	4.90a
R. mucilaginosa	0.994	2.01	4.42b	4.41b	4.65a	4.56a	4.13c	4.56a
S. cerevisiae	0.983	1.92	3.58b	3.01c	3.98a	4.01a	3.19d	3.93a
T. delbrueckii	0.863	0.98	5.64a	5.57b	5.64a	5.61ab	5.59ab	5.58ab
Z. bailii	0.975	2.64	3.70bc	3.70bc	3.59cd	3.84a	3.51d	3.77ab
Z. rouxii	0.992	1.18	5.09ab	4.88c	5.08b	5.16a	4.53d	5.08b
Mean			4.89c	4.86c	4.95ab	4.98a	4.72d	4.92b

[a]Coefficient of variation (repeatability) within each yeast.
[b]Mean values represent composite populations recovered from samples stored at −18°C, 5°C, and 25°C. Mean values in the same row that are followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 6
Mean populations of 13 yeasts recovered on six media after storing for 42 weeks at −18°C, 5°C, and 25°C

Storage temp. (°C)	R^2	CV (%)[a]	Population ($\log_{10}$ cfu/ml)[b]						
			DG18	DRBC	PCAC	TGYC	APDA	OSA	Mean[c]
−18	0.776	7.55	a5.24ab	a5.18ab	a5.27ab	a5.29a	a5.07b	a5.24ab	a5.21
5	0.774	8.76	a5.02a	a5.02a	a5.06a	a5.10a	b4.72a	a5.08a	b5.03
25	0.946	5.76	b4.42ab	b4.39b	b4.12ab	b4.55a	c4.19c	b4.52ab	c4.43
Mean			4.49c	4.86c	4.95ab	4.98a	4.72d	4.95b	

[a] Coefficient of variation (repeatability) within temperature for 13 yeasts.
[b] Population detected in rehydrated suspension. Mean populations of all yeasts recovered on each medium. Values in the same row that are followed by the same letter are not significantly different ($\alpha = 0.05$). Values in the same column that are preceded by the same letter are not significantly different ($P > 0.05$).
[c] Mean population of 13 yeasts recovered on six media.

DG18 and DRBC was significantly better than on APDA but poorer than TGYC, PCAC, and OSA. The poor performance of APDA may be caused by the secondary stresses imposed by its low pH and marginal nutrient availability on yeast cells already stressed by prolonged desiccation.

Listed in Table 6 are composite populations of the 13 test yeasts recovered on six media after storage for 42 weeks at −18°C, 5°C, and 25°C. Significantly higher numbers of cells retained viability at −18°C than at 5°C; in turn, a significantly higher number of cells retained viability at 5°C compared to 25°C. The difference in mean counts of lyophilized yeasts stored at −18°C and 25°C, however, was only 0.78 $\log_{10}$ cfu/ml of rehydrated suspension, indicating a high level of retention of viability unrefrigerated. Decrease in populations in lyophilized cultures stored at 25°C ranged from 0.06 $\log_{10}$ cfu/ml of rehydrated suspension to 1.75 $\log_{10}$ cfu/ml for *T. delbrueckii* and *R. mucilaginosa*, respectively (Table 7).

Table 7
Populations ($\log_{10}$ cfu/ml) of yeasts recovered on six media after storing for 42 weeks at −18°C, 5°C, and 25°C
Population ($\log_{10}$ cfu/ml) detected in rehydrated suspension.

Yeast	Temperature (°C)[a]			
	−18	5	25	Difference[b]
C. krusei	5.04b	5.11a	4.76c	0.28
C. parapsilosis	5.40a	5.35a	4.72b	0.68
Cry. albidus	5.48a	5.40a	4.77a	0.71
D. hansenii	5.70a	5.43b	4.78c	0.71
H. uvarum	4.87a	4.71a	3.83b	0.92
K. marxianus	5.67a	5.53b	5.15c	0.52
P. anomala	5.86a	5.87a	5.73b	0.13
P. membranifaciens	5.43a	5.07b	4.00c	1.43
R. mucilaginosa	5.20a	4.71b	3.45c	1.75
S. cerevisiae	3.85a	3.66b	3.49c	0.36
T. delbrueckii	5.63a	5.61ab	5.57b	0.06
Z. bailii	4.12a	3.71b	3.23c	0.89
Z. rouxii	5.55a	5.24b	4.11c	1.44

[a] Mean values represent composite populations recovered on six media (DG18, DRBC, PCAC, TGYC, APDA, and OSA). Values in the same row that are followed by the same letter are not significantly different ($\alpha = 0.05$).
[b] Difference in population ($\log_{10}$ cfu/ml of rehydrated suspension) in lyophilized cultures stored at −18°C and 25°C for 42 weeks.

Results from both the interlaboratory study and the follow-up storage study indicate that TGYC, PCAC, and OSA are superior to DG18 for recovering yeasts from a desiccated condition. Overall, TGYC performed better than the other five enumeration media. These observations are in agreement with another interlaboratory study (Deak et al., 2001), in which the same strains of actively growing yeasts were plated on DG18, DRBC, PCAC, and TGYC. The use of DG18 for enumerating yeasts stressed by desiccation or unstressed yeasts cannot be recommended.

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