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A collaborative study on media for the enumeration of yeasts in foods

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Abstract

A collaborative study was made to evaluate the effectivity of a general purpose medium, tryptone glucose yeast extract (TGY) agar on the detection and enumeration of yeasts from food. Nine laboratories participated in the study and compared five media (four kinds of TGY with different concentrations of glucose, one of them without tryptone, and, for comparison, dichloran rose bengal chloramphenicol (DRBC) agar). Six food samples were investigated by each laboratory and 23 additional food samples were investigated individually by different laboratories. No difference was found in the performance of media with either the samples common to all laboratories or the various samples tested in different ones. A medium consisting of tryptone, glucose and yeast extract, at any concentration of glucose tested, appeared reliable for the detection and enumeration of yeasts from foods, and its performance did not differ from that of DRBC. Omission of tryptone as recommended by ISO provided an even simpler medium of equally good performance. TGY without chloramphenicol may result in higher total counts due to the development of bacteria. DRBC incubated in light results in lower counts compared to that incubated in the dark.

Keywords: Yeast; Detection; Enumeration; Media; TGY; DRBC

1. Introduction

Several media have been in use for the detection, enumeration and isolation of yeasts from foods (King et al., 1986; Samson et al., 1992; Beuchat, 1992, 1993). For general purposes, tryptone glucose yeast extract (TGY) agar has often been recommended. However, media with different composition, especially with respect to the concentration of glucose, were described under this name (King et al., 1986). In a previous collaborative study (Deak, 1992) TGY agar either acidified (pH 3.5) or supplemented with chloramphenicol (100 mg l^{-1}) was tested. A similar and simpler medium, yeast extract glucose chloramphenicol (YGC) agar is recommended as an ISO standard medium (ISO, 1987) which merits further evaluation. In a number of laboratories the commercial plate count agar, basically a TGY agar with only 1% glucose (Oxoid CM325, Difco 0479) has been in use, and found quite

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satisfactory as a general purpose medium for yeasts. Another TGY agar without antibiotic but with high (10%) glucose concentration is recommended for the mycological examination of foods that contain mainly yeasts (Samson et al., 1992).

In order to clarify this confusing situation, a collaborative study has been initiated by the International Commission of Food Mycology. TGY media of various compositions was tested against the widely used dichloran rose bengal chloramphenicol (DRBC) agar. In addition, the sensitivity of yeasts to rose bengal under light was also addressed.

2. Materials and methods

Nine collaborators participated in the study: L.R. Beuchat (University of Georgia, Griffin, GA, USA), M.E. Guerzoni (University of Bologna, Bologna, Italy), A. Lillie (A. Jorgensen Laboratory, Copenhagen, Denmark), G. Peter (University of Horticulture and Food Science, Budapest, Hungary), H. Rohm (University of Agriculture, Wien, Austria), J. Schnürer (University of Agricultural Science, Uppsala, Sweden), P.V. Tabajdine (State Food Quality Institute, Budapest, Hungary), and S. Westphal (Technical University, Lingby, Denmark).

2.1. Samples

Six coded samples (25 g each) were shipped to each collaborating laboratory. These samples were naturally contaminated with yeasts in low and high population, in most cases accompanied with molds and bacteria. They were of dry condition and distributed in plastic bags so that their microbial load did not change during storage and shipping. In addition to the common samples, laboratories were asked to include further samples of their choice to make up the total number of samples tested to at least 10. In all, 23 different kinds of food, especially fruits, fruit juices, dairy products and spices were examined individually by the laboratories.

2.2. Media

Five media were compared by all laboratories (Table 1). Of these, four represented variations of TGY agar differing in the concentration of glucose. Chloramphenicol was included in all but one, in turn, tryptone was omitted from only one of them. DRBC was prepared according to King et al. (1986). Poured plates were dried overnight before use. Some laboratories, in addition, included for comparison other media such as acidified potato dextrose agar, dichloran 18% glycerol agar, malt extract agar, and oxytetracycline glucose yeast extract agar. These were prepared according to formulas given in the Appendix of Samson et al. (1992).

2.3. Methods

A standard protocol to be followed as to the sample preparation, stomaching, settling, dilution, surface plating, incubation and counting was sent to the collaborating laboratories. In brief, a 10 g sample in duplicate was measured into 90 ml 0.1% peptone water diluent and homogenized in a Stomacher for 2 min. Of this 10^{-1} dilution, after 3 min settling, a serial decimal dilution was prepared in 0.1% peptone water up to 10^{-4} or higher as necessary. From each dilution 0.1 ml was pipetted on the surface of agar plates of each media tested, and spread immediately with a bent glass rod. Plates were incubated in an upright position at 25°C for 5 days.

Table 1
Comparison of main ingredients (%) of media tested

Name	Tryptone	Glucose	Yeast extract	Chloramphenicol
TGY	0.5	10.0	0.5	None
TGYC	0.5	1.0	0.5	0.01
YGC	None	2.0	0.5	0.01
PCAC	0.5	0.1	0.25	0.01
DRBC[a]	0.5	1.0	None	0.01

[a]For other ingredients see King et al. (1986).

2.4. Evaluation

The number of colonies developed on plates were counted after 3 and 5 days. The number of colonies of suspected yeasts, molds and bacteria was recorded separately. In case of doubts, each colony type was checked microscopically in a wet mount preparate. Original data read as CFU per plate at each respective dilution were tabulated and sent to the coordinating laboratory. After expressing in CFU per g and $\log_{10}$ transformation, data were subjected to statistical evaluation by multifactorial analysis of variance using a Statgraphics 5.1 program (Statistical Graphic Corporation, USA).

3. Results and discussion

A total of 430 samples of 29 different kinds of food were examined in the nine laboratories, of them six samples were common. Samples represented low ($\log_{10}$ 0.7 to 2.1) and high ($\log_{10}$ 5.0 to 8.1) CFU g^{-1} of yeasts, and yeasts were generally accompanied with molds and bacteria in the natural contaminating microbiota of foods. The average CFU g^{-1} of yeasts in the common samples ranged from $\log_{10}$ 1.62 to 3.88 (Table 2). Each sample was also contaminated with molds in somewhat lower population than that of yeasts, with the exception of ground coconut which contained only yeasts. Conversely, samples of bran meal contained a higher population of molds than yeasts, whereas only molds were detected in black sunflower seed samples.

The statistical analysis was directed to evaluate the effect of three factors on $\log_{10}$ CFUs of yeasts and molds recovered from samples: the type of food, the laboratories and the media. As expected, statistically significant differences were obtained between samples (not shown) because their yeast and mold loads differed widely and ranged from 0.7 to 8.1 CFU g^{-1}. When laboratories were compared (with all common samples and all media, the total data were 50 for each laboratory with each group of fungi), differences among them proved to be significant (Table 3). Although laboratories were asked to follow a carefully standardized protocol, there appeared significant differences between them concerning the average productivity in detecting yeasts and molds. This was partly due to the outlying performance of Laboratory 1 which detected the smallest CFUs in common samples for both groups of microorganisms.

Contrary to differences in the level of contamination in the samples and to the interlaboratory differences in performance, the comparison of media for the recovery of yeasts and molds from common samples resulted in no statistically significant differences (Table 4). In this case, however, a larger number of data was evaluated (all common samples and all laboratories, a total of 90 data for each medium and each group of fungi) changing the value of least significant difference. Two main conclusions can be drawn from this result: (i) the effectivity of media is independent from the size of population examined, and (ii) neither composition of TGY differ in productivity from DRBC.

These conclusions are corroborated by the results of investigation of individual samples tested by different laboratories. Individual samples differed widely in yeast population ranging from $\log_{10}$ 1.28 to 8.09 CFU g^{-1} (Table 5) and yeast were accompanied with molds and bacteria alike. Comparison of

Table 2
Populations of yeasts and molds ($\log_{10}$ CFU g^{-1}) in common samples examined in all laboratories

Sample	Yeasts			Molds		
	Mean	S.D.	Range	Mean	S.D.	Range
Wheat grains	3.63	0.029	2.74–4.32	2.45	0.071	1.18–4.04
Bran meal	2.06	0.062	1.00–4.04	2.71	0.040	1.00–3.40
Grape pomace	3.83	0.056	2.60–5.08	3.46	0.092	2.15–4.30
Ground coconut	1.62	0.065	0.70–3.54	n.d.		
Sunflower seed, black	n.d.			3.50	0.059	2.15–4.38
Sunflower seed, striated	3.88	0.083	1.00–5.58	3.11	0.092	1.60–4.95

S.D., standard deviation; n.d., not detected in 1 g.

Table 3
Recovery level (in $\log_{10}$ CFU g^{-1}) of yeasts and molds detected in common samples by different laboratories

No. of laboratories	Yeast			Molds		
	Mean	S.D.	Difference	Mean	S.D.	Difference
1	3.03	0.105	a	2.48	0.072	a
2	4.16	0.182	ef	2.98	0.110	bc
3	3.64	0.115	bc	3.41	0.075	e
4	4.21	0.194	f	2.88	0.114	b
5	3.59	0.154	b	3.20	0.104	de
6	3.79	0.179	de	3.31	0.133	de
7	3.86	0.172	cd	3.31	0.103	b
8	3.50	0.127	b	3.19	0.055	cd
9	4.04	0.150	def	3.12	0.093	cd

Data means averages of five media and five samples.
a–f: different letters in the same column indicate significant differences between labs.

Table 4
Comparison of media in the examination of common samples

Media	Yeasts			Molds		
	Mean	S.D.	Range	Mean	S.D.	Range
TGY	3.80	0.113	1.00–5.08	3.04	0.079	1.00–4.95
TGYC	3.77	0.120	1.00–4.72	3.00	0.078	1.18–4.68
YGC	3.81	0.116	1.00–5.58	3.04	0.081	1.48–4.81
PCAC	3.73	0.124	0.70–5.57	3.07	0.079	1.48–4.73
DRBC	3.79	0.119	0.70–5.55	3.08	0.075	1.40–4.69

Data in $\log_{10}$ CFU g^{-1} are averages of five samples in nine laboratories; least significant differences ($P = 0.05$) 0.172 and 0.164 for yeasts and molds, respectively.

Table 5
Populations of yeasts and molds ($\log_{10}$ CFU g^{-1}) in various food samples investigated individually in different laboratories

Food type	No. of different samples	Range of CFUs	
		Yeasts	Molds
Juice concentrates	2	1.41–2.06	n.d.
Apple, grape must	2	2.29–3.39	1.70–2.87
Pickles	3	3.05–4.45	3.13–3.62
Spices	2	5.30–5.67	4.46–6.15
Meat and fish	3	1.28–5.83	1.54–2.03
Spoiled fruit juice	3	4.37–6.29	2.04–2.52
Yoghurt, kefyr	2	4.34–6.32	n.d.
Cheeses	6	3.78–7.50	2.40–8.09

Data are averages obtained on all media tested.

media for the examination of 23 different samples showed a general agreement in yeast and mold counts (Table 6) with the exception of TGY without chloramphenicol. On this medium significantly higher counts of yeasts were obtained than on other media which was most probably due to the error in counting some bacterial colonies for yeasts. In the case of molds, CFUs differed significantly between TGY and DRBC which can be attributed to the partial inhibition of mold development on the latter medium.

Five laboratories compared the effect of incubation in dark and light on the CFUs developed on DRBC. As it was expected from earlier observations (Banks et al., 1985), a toxic derivative of rose bengal inhibited the growth of yeasts and molds on DRBC

Table 6
Comparison of media for the investigation of individual samples

Media	Yeasts				Molds			
	Mean	S.D.	Range	Diff.	Mean	S.D.	Range	Diff.
TGY	4.47	0.128	2.17–7.91	a	4.32	0.078	2.19–8.09	a
TGYC	4.33	0.122	1.43–6.87	b	4.12	0.079	2.02–7.75	ab
YGC	4.28	0.123	1.27–7.08	b	4.17	0.075	2.04–7.60	ab
PCAC	4.32	0.123	1.47–6.97	b	4.25	0.075	1.70–7.73	ab
DRBC	4.27	0.126	1.29–6.42	b	4.09	0.071	1.54–7.08	b

Data in $\log_{10}$CFU g^{-1} are averages of 62 and 28 samples for yeasts and molds, respectively, investigated in five laboratories. Least significant differences ($P = 0.05$) 0.124 and 0.120 for yeasts and molds, respectively.

incubated in light (Table 7), however, the difference against DRBC incubated in dark was only marginally significant in the case of yeasts.

In conclusion, a medium consisting of tryptone, glucose and yeast extract, independently from the concentration of glucose from 0.1 to 10%, can be reliably used for the detection of yeasts from foods with a degree of recovery equal to that of DRBC. Similar results were obtained in an earlier comparative study (Deak, 1992). Omission of tryptone as recommended by ISO (1987) provides an even simpler medium of equally good performance. Omission of chloramphenicol, however, may result in higher total counts due to the development of bacteria even at high (10%) glucose concentration. Corroborating earlier findings, incubation of DRBC in light results in significantly lower yeast counts caused by the inhibition of a toxic derivative of rose bengal.

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Table 7
Comparison of $\log_{10}$ CFUs g^{-1} obtained on DRBC incubated in dark and light

Incubation	Yeasts			Molds		
	Mean	S.D.	Range	Mean	S.D.	Range
Dark	4.41	0.045	2.97–6.90	3.48	0.042	1.89–6.13
Light	4.28	0.045	2.97–6.54	3.38	0.042	1.67–6.11

Averages of 36 different samples from five laboratories. Least significant difference ($P = 0.05$) 0.128 and 0.118, respectively, for yeasts and molds.