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Comparative study of some factors affecting enumeration of moulds using dilution plate techniques

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Summary

The influence of dilution plating technique, nature of diluent, culture media and incubation period on the enumeration of moulds have been studied. Three new culture media containing Auramine, Gentian Violet and Malachite Green respectivelly have been induced in this study. No significant differences were observed between results obtained after 3, 5 and 7 days of incubation. Significantly higher recoveries were obtained using the surface-spread method than pour plate method. Using the first technique no effect of diluent was observed, and among the different culture media studied higher counts were obtained with medium containing Auramine.

Key words: Culture media, diluents, dyes, fungal enumeration, incubation period.

Resumen

Se ha analizado la influencia de la técnica de siembra en placa, la naturaleza del diluyente, el medio de cultivo y el tiempo de incubación sobre el recuento de hongos miceliares. Se han incluido tres nuevos medios de cultivo conteniendo Auramina, Violeta de Genciana y Verde de Malaquita, respectivamente. No han aparecido diferencias significativas entre los resultados obtenidos después de 3, 5 y 7 días de incubación. Los recuentos obtenidos mediante la técnica de siembra en superficie de agar han resultado significativamente superiores que los de la técnica por inclusión en agar. Utilizando la primera de estas técnicas no se ha observado efecto del diluyente empleado y mediante el medio de cultivo adicionado de Auramina se han obtenido recuentos fúngicos más altos.

Introduction

Nature and extent of fungal contamination are important criteria in judging hygiene and storage practices during the manufacture and distribution of food. The detection and quantification of fungi is therefore an essential part of the microbiological examination of foods. Many different methods are available: cultural, direct microscopic and indirect methods (8). When only one metho-

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dology is followed, every attempt should be made to ensure the maximum recovery of organisms under investigation.

The cultural methods allow detection of viable fungus material, even at relatively low contamination levels and among them, the most used are the colony count techniques. As in bacteriological analysis, the principle behind the propagule count is the preparation of sample homogenate, followed by serial dilution in an appropriate diluent and plating onto or into appropriate culture media. There are several factors which can affect results of propagule counts such as plating technique, nature of diluent, culture media and incubation period. To evaluate the influence of these factors on the enumeration of moulds, an experimental study has been designed to compare the recovery of the tested strains by the surface and pour-plate methods seven culture media, including three new media containing auramine, gentian violet and malachite green, respectivelly. The effect of incubation time has been evaluated comparing the results obtained after 3, 5 and 7 days of incubation.

Strains

The moulds used in this study were: *Aspergillus parasiticus* NRRL 2999, *Penicillium verrucosum* var. *cyclopium* CCFVB 417 (CCFVB denotes the Culture Collection of the Faculty of Veterinary of Barcelona), *Fusarium oxysporum* CCFVB 300 and *Alternaria alternata* CCFVB 252. These strains have been selected because of their high frequency of isolation in substrata such as foodstuffs and/or their ability to produce mycotoxins. The mould suspensions were prepared from cultures grown separately on 2 % Malt Extract Agar for seven days at 28° C. After incubation, spores were harvested by adding 10 ml of distilled water to the culture vessels and gently dislodging the spores with a flamed wire loop. The spore suspensions were then aseptically filtered through sterile gauze to remove mycelial debris, and the volumes adjusted so that the suspensions contained approximately 10^5 spores/ml as determined by a counting chamber. A mixed-spore suspension was prepared taking equal volumes of each individual spore suspension.

Diluents

A serial dilution of the mixed spore-suspension was made in the following five diluents: Tryptone water (TW), Distilled water (DW), Phosphate buffered saline (PBS), Saline water (0.90 % v/v) (SW), and Saline water + 0.05 % Tween 80 (SWT).

Culture media

Seven media (final pH, 5.6 ± 0.2) were tested for recovery of moulds from the mixed-spore suspension: Malt extract agar (MEA), containing (g/l): malt extract, 13; Dextrin, 2.5; Peptone, 5 and agar, 15; Sabouraud Dextrose agar (S) containing (g/l): Peptone, 10; Dextrose, 40 and agar, 15; MEA + 50 ppm of Rose Bengal (RB); MEA + 2 ppm of Dichloran (D); MEA + 25 ppm of Auramine (A); MEA + 5 ppm of Gentian Violet (GV) and MEA + 1 ppm of Malachite Green (MG).

Colony count techniques

Quantitative enumeration of fungal propagules was done on solid media using the pour plate and surface-spread method. Five plates were inoculated for each diluent and medium used.

TABLE 1

MEAN VALUES OF TOTAL COUNTS IN CFU/ml ($x10^{-4}$) OBTAINED BY THE SURFACE AND POUR-PLATE METHODS, USING DIFFERENT CULTURE MEDIA, DILUENTS AND INCUBATION TIMES

M	DIL	Surface-spread			Pour-plate		
		Incubation time (days)			Incubation time (days)		
		3	5	7	3	5	7
MEA	TW	46.0	46.0	46.0	41.0	41.0	41.0
	DW	50.4	50.6	50.6	36.4	36.4	36.4
	PBS	57.2	57.2	57.2	40.2	40.2	40.2
	SW	48.6	48.6	49.6	40.8	40.8	40.8
	SWT	55.8	56.2	56.2	46.6	46.6	46.6
S	TW	38.0	38.0	38.0	38.8	38.8	38.8
	DW	42.8	43.4	43.4	37.2	37.8	37.8
	PBS	46.2	46.2	46.2	42.8	43.6	43.6
	SW	58.0	58.4	58.4	38.8	39.4	39.4
	SWT	50.2	50.5	50.7	29.8	29.8	29.8
A	TW	56.6	56.8	56.8	37.8	37.8	37.8
	DW	55.0	55.0	55.0	47.6	48.0	48.0
	PBS	55.6	55.6	55.6	44.0	44.0	44.0
	SW	53.4	54.0	54.0	45.6	45.6	45.6
	SWT	62.8	62.8	62.8	53.4	53.4	53.4
D	TW	45.6	46.6	46.6	35.4	35.4	35.4
	DW	50.8	51.2	51.2	35.4	35.4	35.4
	PBS	47.8	47.8	47.8	35.2	35.2	35.2
	SW	55.4	55.8	55.8	38.5	38.5	38.5
	SWT	53.2	53.7	53.7	51.0	51.0	51.0
RB	TW	54.6	56.0	56.0	43.6	43.6	43.6
	DW	50.6	52.6	52.6	20.0	20.0	20.0
	PBS	50.2	56.2	56.2	44.7	44.7	44.7
	SW	48.8	51.8	51.8	42.4	42.6	42.6
	SWT	51.4	52.8	52.8	63.8	64.6	64.6
GV	TW	40.0	40.6	41.2	36.8	36.8	36.8
	DW	33.8	38.0	38.0	31.6	31.6	31.6
	PBS	33.0	34.4	34.4	33.0	33.0	33.0
	SW	38.8	41.4	42.4	38.8	38.8	38.8
	SWT	39.0	39.2	39.6	41.2	41.6	41.6
MG	TW	47.8	48.8	48.8	35.6	36.2	36.2
	DW	39.6	47.4	47.4	25.2	25.6	25.6
	PBS	44.0	47.6	47.6	39.6	40.0	40.0
	SW	42.0	44.6	46.2	33.0	33.2	33.2
	SWT	45.2	47.2	47.2	31.2	31.4	31.4
	x	48.3	49.5	49.6	39.3	39.5	39.5
	δ_{n-1}	11.1	10.8	10.7	9.5	9.5	9.5

M: culture media; DIL: diluents. MEA: Malt Extract Agar; S: Sabouraud Dextrose Agar; A: MEA + 25 ppm of Auramine; D: MEA + 2 ppm of Dichloran; RB: MEA + 50 ppm of Rose Bengal; GV: MEA + 5 ppm of Gentian Violet; MG: MEA + 1 ppm of Malachite Green. TW: Tryptone water; DW: Distilled water; PBS: Phosphate buffered saline; SW: Saline water; SWT: Saline water + 0.05% Tween 80.

TABLE 2
SUMMARY OF p VALUES FROM THE ANALYSIS OF VARIANCE OF TOTAL COUNT RESULTS

Main effects			Interactions			
Technique	(T)	**	T-M	**	T-M-D	**
Medium	(M)	**	T-D	**	T-M-t	n.s.
Diluent	(D)	**	T-t	n.s.	T-D-t	n.s.
Time	(t)	n.s.	M-D	**	M-D-t	n.s.
			M-t	n.s.		
			D-t	n.s.		

**: $p < 0.01$; n.s.: not significant.

a) *Surface-spread method:* Spread plates were prepared by spreading 0.1 ml aliquots of each dilution tested on surface-dried agar plates using a sterile, bent, glass rod. Disposable 9.0 cm Petri dishes containing 20 ml of media were used.
b) *Pour plate method:* Pour plates were prepared by delivering 1 ml aliquots of each dilution level tested into disposable 9.0 cm Petri dishes, adding 20 ml of the molten medium at 45° C and mixing at a room temperature.

Plates were incubated at 28° C for seven days. Only plates with 10-100 colony forming units (CFU) were used for counting. Total CFU/ml was determined after 3, 5 and 7 days of incubation. In the media inoculated using the surface-spread method, number of CFU/ml for each fungal species inoculated (partial counts) were also recorded after 5 and 7 days of incubation.

Results

Table 1 shows the mean value of total mould counts after 3, 5 and 7 days of incubation using the pour plate and the surface-spread methods, the five diluents and the seven culture media studied.

Data were subjected to analysis of variance, studying the main effects (method, culture medium, diluent and incubation time) and their interactions (2-way and 3-way interactions). The p value for each test and significant results are noted in Table 2. As no differences were observed between incubation time, the other three variables were studied using the results of mould counts obtained after three days of incubation.

The effect of method on recovery of moulds was determined by Student's t test. Significant

TABLE 3
SUMMARY OF p VALUES FOR THE ANALYSIS OF VARIANCE OF TOTAL COUNT RESULTS OBTAINED WITH SURFACE-SPREAD TECHNIQUE AND THE POUR PLATE TECHNIQUE

Technique	Medium	Diluent	Medium-diluent
Surface-spread	**	n.s.	n.s.
Pour-plate	**	**	**

**: $p < 0.01$; n.s.: not significant.

TABLE 4
EFFECT OF CULTURE MEDIUM ON RECOVERY OF MOULDS USING THE SURFACE SPREAD METHOD (NEWMAN-KEULS TEST)

x		GV	MG	S	D	RB	MEA	A
36.92	GV							
43.72	MG	*						
46.91	S	*						
50.45	D	*	*					
51.56	RB	*	*					
51.83	MEA	*	*					
56.86	A	*	*	*				

x: mean counts in CFU/ml (x 10^{-4}). *: $p < 0.05$.

differences were recorded ($p < 0.01$) due to the fact that the surface inoculated plates yield higher counts (x = 48.3) than the pour plates (x = 39.3).

As shown in Table 3, using the surface-spread technique, no statistical differences were recorded neither between diluents nor in the interaction medium-diluent. The significant differences observed between media ($p < 0.01$) were analysed by the Newman-Keuls test (Table 4).

Using the pour plate technique, significant differences ($p < 0.01$) were observed between media and diluents. It was not possible to determine which was the best medium or diluent due to a significant interaction ($p < 0.01$) between medium and diluent (Table 3). Taking in account the possible combinations medium-diluent, the Newman-Keuls test showed that RB with SWT achieved significantly higher counts (x = 63.8). Colony counts obtained with A medium and SWT were also high (x = 53.4), but no significantly different to those obtained with the combinations D-SWT, A-DW, MEA-SWT, A-SW, RB-PBS, RB-TW, S-PBS and RB-SW.

The partial counts for each inoculated species using the surface spread method are shown in Table 5. No differences were observed between results obtained after five and seven days of incubation, so the effect of culture medium and diluent were studied using results obtained after five days of incubation. The p value and significant results obtained by the analysis of variance are noted in Table 6. The significant differences were then analysed by the Newman-Keuls test except when there was not homogeneity of variance (*P. verrucosum* var. *cyclopium* and *A. alternata*). Results are shown in Table 7.

Discussion

Some Microbiological Manuals (12, 13, 15, 16) and several authors (10, 17) recommend five days minimum for the mould count. Zipkes *et al.* (17) reported that mould counts were significantly higher after 5 days than those achieved after 3 days, but they did not find significant changes in yeast counts between 3 and 5 days incubation data. From the results obtained in the present study, it may be deduced that mould counts are not affected by the incubation time. Therefore, it is possible to obtain a result after three days of incubation, according to the recommendation made by Janes and Tilbury (6).

The plating method may be also effect to the viable mould count. The pour-plate method is the technique recommended in the Microbiological Standard Methods for enumeration of fungi (12, 13, 15, 16). Zipkes *et al.* (17) made a comparison of yeast and mould counts by spiral, pour and

TABLE 5

PARTIAL COUNTS IN CFU/ml (x 10^{-4}) OF THE EACH INOCULATED STRAIN OBTAINED BY THE SURFACE-SPREAD METHOD USING DIFFERENT CULTURE MEDIA, DILUENTS AND INCUBATION TIME

M	DIL	Incubation time (days)							
		5	7	5	7	5	7	5	7
MEA	TW	14.2	14.2	6.5	6.5	21.5	21.5	3.7	3.7
	DW	17.2	17.2	8.2	8.2	18.6	18.6	4.4	4.4
	PBS	16.0	16.0	10.6	10.6	22.0	22.0	9.0	9.0
	SW	16.6	17.6	6.0	6.0	20.2	20.2	5.8	5.8
	SWT	18.0	18.0	10.8	10.8	20.2	20.2	7.4	7.4
S	TW	14.2	14.2	8.6	8.6	13.0	13.0	2.2	2.2
	DW	13.6	13.6	10.6	10.6	15.6	15.6	3.2	3.2
	PBS	19.8	19.8	9.0	9.0	14.0	14.0	5.4	5.4
	SW	27.4	27.4	9.2	9.2	14.4	14.4	7.2	7.2
	STW	14.5	14.7	8.0	8.0	20.7	20.7	7.2	7.2
A	TW	18.8	18.8	7.8	7.8	25.6	25.6	6.6	6.6
	DW	21.4	21.4	8.0	8.0	20.6	20.6	5.0	5.0
	PBS	17.8	17.8	8.4	8.4	23.4	23.4	6.0	6.0
	SW	16.0	16.0	6.8	6.8	22.2	22.2	9.0	9.0
	STW	19.4	19.4	9.4	9.4	27.4	27.4	6.6	6.6
D	TW	18.8	18.8	4.6	4.6	18.6	18.6	4.4	4.4
	DW	18.4	18.4	13.8	13.8	13.8	13.8	5.0	5.0
	PBS	15.8	15.8	6.8	6.8	19.2	19.2	6.0	6.0
	SW	17.6	17.6	7.4	7.4	23.2	23.2	7.4	7.4
	STW	19.4	19.4	6.6	6.6	20.0	20.0	7.6	7.6
RB	TW	21.8	21.8	7.0	7.0	23.2	23.2	4.2	4.2
	DW	23.0	23.0	7.4	7.4	18.6	18.6	4.2	4.2
	PBS	24.0	24.0	6.0	6.0	20.4	20.4	5.8	5.8
	SW	20.0	20.0	6.2	6.2	23.0	23.0	5.0	5.0
	STW	15.0	15.0	9.2	9.2	21.0	21.0	6.4	6.4
GV	TW	0.0	0.0	5.8	5.8	29.6	29.6	5.2	5.8
	DW	0.0	0.0	8.2	8.2	25.0	25.0	4.8	4.8
	PBS	0.0	0.0	2.4	2.4	25.4	25.4	6.6	6.6
	SW	0.0	0.0	3.6	3.6	27.8	27.8	10.0	10.4
	STW	0.0	0.0	4.8	4.8	28.6	28.6	5.8	6.0
MG	TW	11.2	11.2	9.4	9.4	26.2	26.2	2.0	2.0
	DW	7.6	7.6	11.6	11.6	22.4	22.4	2.4	2.4
	PBS	15.4	15.4	4.4	4.4	21.8	21.8	6.0	6.0
	SW	8.4	8.4	4.6	4.6	28.8	28.8	2.8	4.4
	STW	12.8	12.8	7.6	7.6	24.4	24.4	3.8	3.8

M: culture media; DIL: diluents. MEA: Malt Extract Agar; S: Sabouraud Dextrose Agar; A: MEA + 25 ppm of Auramine; D: MEA + 2 ppm of Dichloran; RB: MEA + 50 ppm of Rose Bengal; GV: MEA + 5 ppm of Gentian Violet; MG: MEA + 1 ppm of Malachite Green. TW: Tryptone water; DW: Distilled water; PBS: Phosphate buffered saline; SW: Saline water; SWT: Saline water + 0.05% Tween 80.

TABLE 6
ANALYSIS OF VARIANCE OF PARTIAL COUNTS RESULTS

	Medium	Diluent	Medium-diluent
P. verrucosum	**	n.s.	n.s.
A. parasiticus	**	**	n.s.
F. oxysporum	**	n.s.	n.s.
A. alternata	n.s.	*	n.s.

**: $p < 0.01$; *: $p < 0.05$; n.s.: not significant.

TABLE 7
EFFECT OF CULTURE MEDIUM ON RECOVERY OF *A. PARASITICUS* AND *F. OXYSPORUM*, AND EFFECT OF DILUENT ON RECOVERY OF *A. PARASITICUS* (NEWMAN-KEULS TEST)

A. parasiticus

x		GV	RB	MG	D	A	ME	S
4.96	GV							
7.16	RB							
7.76	MG	*						
7.95	D	*						
8.08	A	*						
8.50	ME	*						
9.12	S	*						

F. oxysporum

x		S	D	ME	RB	A	MG	GV
15.46	S							
18.95	D	*						
20.87	ME	*						
21.28	RB	*	*					
23.84	A	*	*					
25.32	MG	*	*	*	*			
27.28	GV	*	*	*	*			

A. parasiticus

x		SW	PBS	TW	SWT	DW
6.25	SW					
6.80	PBS					
7.11	TW					
8.24	SWT					
9.77	DW	*	*	*		

x: Mean partial counts in CFU/ml (x 10^{-4}). *: $p < 0.05$.

spread plate methods, obtaining higher recoveries by the spiral and spread methods. Pitt and Hocking (14) reported that spread plating is generally considered to be a more suitable technique for dilution plating than the pour plate method. Although other authors (8, 10) are in disagreement with this opinion. In our case, the spread plate technique gave significantly higher counts than the pour plate method. Although choice of method is related to a particular set of circumstances and may not exist a method which satisfies all the requirements, the pour plate method may present some disadvantages as the difficulty of isolation of colonies and their identification to at least the genus level. Moreover, the termal sensitivity of mould propagules can lead to a reduction in colony counts (3) and the volume of agar also can reduce pour plate propagule counts, presumably by reducing the oxygen tension (8).

The third factor analysed has been the nature of diluent. Although no differences have been obtained with the five diluents studied using the surface spread method, higher recoveries were obtained with SWT, followed by SW and PBS. Taking in account the effect due to diluent in the pour plate method, higher counts were obtained with SWT, PBS and SW. In the bibliographical review carried out no systematic investigation has been found of the effect of diluent on propagule counts, but in accordance with our results, some authors (8) mentioned that the presence of a wetting agent, such as Tween 80, can be beneficial to increase recoveries of moulds.

The last factor studied has been the culture media. Ideally, culture media for isolating and enumerating moulds should support recovery of all viable propagules. In addition, media should restrict the growth of spreading moulds, inhibit the development of bacterial colonies, and aid in identification of fungi to at least genus level. The effect of some substances such as acids, antibiotics, fungicides and dyes on the growth and counts of moulds have been studied by several authors (1, 2, 5, 7, 9, 11). In the present study, the suitability of two general purpose media as Malt Extract agar and Sabouraud Dextrose agar, as well as five media containing dyes were compared. The use of media containing Rose Bengal and Dichloran have been reported by many authors (1, 4, 5, 7). In preliminary studies (2), the effect of several dyes on colony diameter and enumeration of various fungi (Deuteromycetes and Zygomycetes) were investigated. From the results obtained we selected Auramine, Gentian Violet and Malachite Green because it was observed that Malt Extract agar containing 25 ppm of Auramine, 5 ppm of Gentian Violet and 1 ppm of Malachite Green respectively performed similarly as media containing 50 ppm of Rose Bengal (7) or 2 ppm of Dichloran (9). So, we decided to include these three new culture media in the comparative study reported here. Using the surface spread method, media containing 25 ppm of Auramine yield higher counts although they were no signifficant differences from those obtained using MEA, RB and D. Using the pour plate method, higher recoveries were obtained with A, RB and MEA media.

The colony-forming units of each inoculated species by the surface spread method were determined after 5 and 7 days of incubation and no significant differences were observed. Partial counts were not made after 3 days because growth and sporulation of fungal strains were very scarce, so it was necessary to increase the incubation time to minimum five days. This study was not performed using the pour plate method because it was very difficult to distinguish the different genera growing into the culture media. As shown in Table 5, significant differences were observed between culture media in partial counts of *P. verrucosum* var. *cyclopium*, *A. parasiticus* and *F. oxysporum*. There was no homogeneity of variance in counts of *P. verrucosum* var. *cyclopium*, but higher counts were obtained using RB, A, S, D and MEA without statistical differences between them. This species was inhibited in media containing Gentian Violet and the lowest recovery of *A. parasiticus* was obtained in this media (GV). In contrast, GV, MG and A media yield higher counts of *F. oxysporum*. The fact that Gentian Violet increased counts of this *Fusarium* species was observed in previous studies in our laboratory. Colony counts of *A. alternata* were not affected by the media used, but higher recovery was obtained with A, followed by GV, D, MEA, RB, S and MG, in decreasing order.

The effect of diluent was observed in partial counts of *A. parasiticus* and *A. alternata.* For the first strain, higher counts were recorded using DW and SWT. Although there was not homogeneity of variance in the case of *A. alternata,* higher recoveries were obtained in decreasing order with SW, SWT, PBS, DW and TW.

After consideration of all media developed for enumerating fungi, one has to conclude that one ideal medium does not exist because it is difficult that it contain all the attributes necessary to become the one general purpose medium for fungi. From the results obtained in the present work, it seems that medium containing Auramine is satisfactory for growth and enumeration of mould propagules because it allows equivalent or overall recovery of mould counts, especially those of slower growing fungi.

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References

1. Bothast, R. J. and Fennell, D. I. (1974). A medium for rapid identification and enumeration of *Aspergillus flavus* and related organisms. Mycologia **66,** 365-369.
2. Bragulat, M. R., Abarca, M. L., Bruguera, M. T. and Cabañes, F. J. (1991). Dyes as fungal inhibitors: Effect on colony diameter. Appl. Environ. Microbiol. **57,** 2777-2780.
3. Flanningan, B. (1973). An evaluation of dilution plate methods for enumerating fungi Lab. Pract. **22,** 530-531.
4. Henson, O. E. (1981). Dichloran as an inhibitor of mold spreading in fungal plating media: Effects on colony diameter and enumeration. Appl. Environ. Microbiol. **42,** 656-660.
5. Hocking, A. D. and Pitt, J. I. (1980). Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. Appl. Environ. Microbiol. **39,** 488-492.
6. Janes, L. and Tilbury, R. H. (1981). A comparison of media and methods for the enumeration of yeasts and moulds in refined sugar products. J. Appl. Bacteriol. **51,** p. XIV.
7. Jarvis, B. (1973). Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in foods. J. Appl. Bacteriol. **36,** 723-727.
8. Jarvis, B., Seiler, D. A. L., Ould, A. J. L. and Williams, A. P. (1983). Observations on the enumeration of moulds in food and feedingstuffs. J. Appl. Bacteriol. **55,** 325-336.
9. King, A. D., Hocking, A. D. and Pitt, J. I. (1979). Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl. Environ. Microbiol. **37,** 959-964.
10. Koburger, J. A. and Norden, A. R. (1975). Fungi in foods. VII. A comparison of the surface, pour plate, and most probable number methods for enumeration of yeasts and molds. J. Milk Food Techol. **38,** 745-746.
11. Mossel, D. A. A., Visser, M. and Mengerink, W. H. J. (1962). A comparison of media for the enumeration of moulds and yeasts in foods and beverages. Lab. Pract. **11,** 109-112.
12. Official Methods of Analysis (1984). 14th Ed., AOAC, sec. 46.011, p. 941. Arlington.
13. Pascual, M. R. (1982). Técnicas para el análisis microbiológico de alimentos y bebidas. Ministerio de Sanidad y Consumo. Centro Nacional de Alimentación y Nutrición. Servicio de Microbiología (CNAN). Madrid.
14. Pitt, J. I. and Hocking, A. D. (1985). Fungi and food spoilage. Academic Press. Sidney.
15. Saco, M., Díaz, M. A. and San Gabriel, A. (1986). Microbiología de materias primas para piensos compuestos y otros alimentos animales. Ministerio de Agricultura, Pesca y Alimentación. Madrid.
16. Standard Methods for the examination of Dairy Products (1985). 15th Ed. Richardson, G. H. Chap. 9.8, p. 197. Washington.
17. Zipkes, M. R., Gilchrist, J. E. and Peeler, J. T. (1981). Comparison of yeast and mold counts by spiral, pour, and streak plate methods. J. Assoc. Off. Anal. Chem. **64,** 1465-1469.