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A COMPARISON OF THE ABILITIES OF 15 MEDIA TO RECOVER VIABLE YEASTS FROM POND, RIVER, AND LAKE WATER

by

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MANAGEMENT PERSPECTIVE

Yeasts can be used as indicators of water quality. A variety of agar media have been used in association with the membrane filtration technique (MF), to determine yeast levels in aquatic environments. However, no study has yet been made of the effects, if any, that differences in media composition can have on yeast population estimates. Consequently, the abilities of 15 agar media to resuscitate yeasts from pond, river and lake water were compared. The study was undertaken as part of a wider NWRI project for the development of improved microbial indicators of water pollution as a means towards enhancing the department's ability to cost effectively monitor water quality.

The results indicate that the selection of optimal media for the enumeration of yeasts should be made for individual water bodies. Specific improvements to present yeast enumeration techniques are recommended; these should lead to an improved ability to use yeasts as indicators of water quality.

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PERSPECTIVE - GESTION

Les levures peuvent être utilisées comme indicateurs de la qualité de l'eau. Diverses géloses ont été utilisées avec la technique de filtration par membrane (FM) afin de doser la teneur en levure dans les milieux aquatiques. Cependant, aucune étude n'a encore été faite sur les effets que peuvent avoir les différences dans la composition des milieux sur les estimations de populations de levures. On a donc comparé la capacité de 15 géloses à ressusciter les levures de l'eau des étangs, des rivières et des lacs. Cette étude a été entreprise dans le cadre d'un projet plus vaste de l'INRE portant sur le développement d'indicateurs microbiens de la pollution de l'eau comme moyen d'améliorer la capacité du ministère à vérifier de façon rentable la qualité de l'eau.

Les résultats indiquent que le choix du meilleur milieu pour la numération des levure est particulier à chaque plan d'eau. Des améliorations bien précises des techniques de numération des levures sont donc recommandées. Celles-ci devraient permettre une meilleure utilisation des levures comme indicateurs de la qualité de l'eau.

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ABSTRACT

Membrane filtration (MF) is widely used for the recovery and enumeration of yeasts from fresh water. In the presented study, the abilities of 15 media to recover viable yeasts from pond, river and lake water were compared. The type of water sample filtered appeared to influence the abilities of the media to resuscitate yeasts. Media containing glucose that were also rich in peptone, yeast extract, and malt extract tended to recover the most yeasts; however, no medium was clearly superior for all the water samples. Thus selection of optimal media may have to be undertaken for individual water bodies. The results indicated that some nutrient rich media were susceptible to mould overgrowth; whereas low nutrient media were generally not. Elimination of yeast counts from filters that had $\geq$ 25% of their surface area obscured by spreading moulds led to increased yeast population estimates, which suggests that increased sample replication followed by the rejection of yeast counts obtained from filters that have $\geq$ 25% of their surface obscured by mould overgrowth, could help to limit the influence of spreading moulds on yeast population estimates.

RÉSUMÉ

On utilise beaucoup la filtration par membrane (FM) pour la récupération et la numération des levures présentes dans l'eau douce. La présente étude a comparé les capacités de 15 milieux différents à récupérer les levures viables de l'eau des étangs, des rivières et des lacs. Le type d'échantillon d'eau filtrée semblait influer sur les capapacités du milieu à ressusciter les levures. Les milieux contenant du glucose et riches en peptone, en extrait de levure et extrait de malt avaient tendance à récupérer les plus grandes quantités de levures; cependant, aucun milieu n'était nettement supérieur pour tous les échantillons d'eau. Il faut donc choisir le meilleur milieu pour chaque plan d'eau individuel. Les résultats indiquent que certains milieux riches en substances nutritives étaient susceptibles d'amorcer la prolifération de moisissures, contrairement aux milieux à faible teneur en substances nutritives. On n'a pas tenu compte des numérations de levures dans le cas des filtres dont la surface était couverte à 25 % et plus par les moisissures, ce qui a entraîné des estimations accrues des populations de levures; cela suppose qu'une réplication accrue des échantillons suivie du rejet des numérations de levures obtenues des filtres dont la surface était couverte à 25 % et plus par des moisissures pourrait permettre de limiter l'influence de la prolifération des moisissures sur les estimations des populations de levures.

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1.0 INTRODUCTION

The development of new and improved indicators of water pollution would facilitate the routine and cost effective determination of water quality. This would be advantageous for the surveillance and monitoring of various aquatic ecosystems such as large water bodies and waters that are bordered by heavily populated, or industrialized land, since it is often necessary to analyze large numbers of samples from such systems on a regular basis. Yeasts have been consistently recovered from a variety of aquatic ecosystems (Ahearn et al., 1968; Cook, 1970; Hedric et al., 1966; Simard & Blackwood, 1971); and have been suggested as potential indicators of water quality (Meyers et al., 1970; Woolett & Hedrick, 1970).

Membrane filtration (MF) has been extensively used for the isolation and enumeration of yeasts from marine and freshwater (Sherry and Qureshi, 1981). The MF technique involves passing a water sample through a membrane filter which is then implanted on an agar based nutrient medium and incubated to promote yeast colony development. A variety of agar media, some of which are listed in Table 1, have been so used to determine yeast levels in aquatic environments. However, because agar media may vary in their ability to resuscitate yeasts, their use could bias estimates of yeast levels in water samples. Such erroneous estimates could become important if yeasts were to be used as indicators of pollution or ecological perturbations in aquatic systems. No study has yet been made of the relative abilities of the many available media to recover yeasts from water. Such information would facilitate the selection or design of optimal media for use in investigations of yeast distributions in aquatic environments.

In the present study, which is an initial attempt to address the foregoing problem, the abilities of 15 media to recover viable

yeasts from freshwater are compared. Pond, river and lake water samples were analyzed in order to determine whether media performance is affected by water type.

2.0 METHODS

2.1 Sample Collection

In late autumn, a sub-surface (1 m) water sample (25 L) was collected from each of three locations: from Lake Ontario close to the Burlington Ship Canal; from the Grand River above the town of Caledonia, Ontario; and from a man-made pond containing mesotrophic water that had been originally taken from nearby Lake Ontario. The samples were transferred to sterile containers and stored on ice during transportation to the laboratory.

2.2 Test Procedure

All samples were filtered within 24 h of sample collection. Five, 30-, and 70-mL sample portions were filtered through 0.45 µm gridded membrane filters (Gelman, 47 mm diam) using a procedure described by Dutka (1978). After the simultaneous filtration of two sample portions, the membrane filters were individually implanted on plates of medium #1 (Table 1); filter pairs were similarly implanted on each of the remaining media in sequence. The forgoing procedure was repeated four times for each sample, to give 10 replicates per medium. Yeast colonies were enumerated after five days incubation at 15°C. The percentage of the filter surface obscured by spreading moulds was deduced from a count of the number of mould obscured rectangles per membrane.

3.0 DATA ANALYSIS

Filters that had <25% of their surface area covered by spreading moulds were classed as 'non-obscured', whereas, filters that had $\geq$ 25% of their surface area so covered were classed as 'obscured'. Yeast colony counts were not recorded from filters that were totally obscured.

Inter-media statistical comparisons were made using the appropriate t-test, after a preliminary test of the sample variances for homogeneity. Media ranks (which do not have an implied statistical significance) were compared using Spearman's rank correlation coefficient. ANOVA refers to the appropriate analysis of variance test. The Mann-Whitney test was used for inter-media comparisons of the degree of surface area obscuration.

4.0 RESULTS

4.1 Lake Water

The data for the Lake Ontario water sample (Table 2) indicate that rejection of yeast colony counts from mould obscured membrane filters led to higher average yeast colony counts ($P < 0.05$). Before elimination of the yeast colony counts from the mould overgrown filters (Table 3a), medium #4 appeared to have recovered the most yeast colonies. Upon elimination of the yeast colony counts from the mould obscured filters (Table 3b), medium #2, 40% of whose filters were uncountable, also performed better than the other media with the exception of medium #1. The media performance ranks obtained using both enumeration procedures were correlated ($RS = 0.74$; $P = 0.03$).

Spreading moulds were a significantly greater problem on medium #6 than on 57% of the other media (Table 10a). Table 10a also indicates that most of the media did not differ significantly from each other in their susceptibility to mould overgrowth.

4.2 River Water

The data for the Grand River water sample (Table 4), indicate that rejection of yeast colony counts from mould obscured membrane filters led to higher average yeast colony counts ($P < 0.005$). Before elimination of the yeast colony counts from the mould overgrown filters (Table 5a), medium #4 appeared to have recovered the most, and medium #9 the fewest yeast colonies, with the exception of medium #15 which also performed poorly. Upon elimination of the yeast colony counts from the mould obscured filters (Table 5b), medium #4 again performed better than the other media; whereas, media #15 and #9 recovered fewer yeast coloies than the other media, with the exception of medium #5. The media performance ranks obtained using both enumeration procedures were correlated ($RS = 0.85$; $P = 0.0005$).

Spreading moulds were a significantly greater problem on medium #2 than on 36% of the other media (Table 10b). Table 10b also shows that most of the media did not differ significantly from each other in their susceptibility to mould overgrowth.

4.3 Pond Water

The data for the 5 mL pond water samples (Table 6), indicate that rejection of yeast colony counts from mould obscured membrane filters led to a barely significant increase in average yeast colony counts ($P < 0.05$, $t = 1.762$, signficant at $t = 1.,761$). Analysis of the media performance data before and after elimination of the yeast counts from the mould overgrown filters indicated that medium #2 performed better than the other media, with the exception of medium #13 (Table 7). The media performance ranks for both enumeration procedures were correlated ($RS = 1.00$, $P < 0.01$).

Spreading moulds were a greater problem on media #12 and #11 than on 86% and 50%, respectively, of the other media (Table 10c). Table 10c also indicates that most of the media did not differ

significantly from each other in their susceptibility to mould overgrowth.

The data obtained for the 30 mL pond water samples (Table 8) indicate that rejection of yeast colony counts from mould obscured membrane filters led to a significant increase in average yeast colony counts ($P < 0.05$). Unfortunately, yeast colony merging rendered the filters implanted on media #1, #2, and #4 uncountable. Examination of the media performance data before elimination of the counts from the mould overgrown filters (Table 9a) indicated that media #13, #3, and #15 appeared to perform better than the other media with the exception of medium #8, whereas medium #11 recovered the fewest yeast colonies, with the exception of medium #14.

Upon elimination of the yeast counts from the mould overgrown filters (Table 9b), media #8, #13, #3 and #15 performed better than the other media, with the exception of medium #10 in the case of the latter two media. The media performance ranks obtained using both enumeration procedures were correlated ($RS = 0.95$; $P = 0.001$). The data presented in Table 10d indicate that medium #11, which recovered yeasts poorly, had more of its surface area obscured by moulds than 45% of the other media. Table 10d also shows that significant differences were detected in the extent of surface area obscuration by spreading moulds for 48% of the inter-media comparisons. Medium #3 was less affected by mould overgrowth than the other media, with the exception of media #9 and #7.

The yeast recovery data obtained from the pond water samples (Table 7 and 9) indicate that some inter-media differences may become more pronounced as the volume of the sample filtered, and consequently the number of yeast colonies grown on each filter, increases. The media performance ranks for the 5 mL and 30 mL pond water samples were not significantly correlated after the colony counts from the uncountable filters had been eliminated, but they were correlated ($P = 0.01$) before the elimination of these data.

4.4 Spreading Moulds

The rejection of yeast colony counts from membrane filters that had $\geq$ 25% of their surface area obscured by spreading moulds resulted in significantly higher yeast counts for the river, pond (30 mL samples), and lake water samples. The apparent relationship between the number of uncountable filters and the enhancement of yeast colony counts through the elimination of counts obtained from mould obscured filters is substantiated by the data presented in Figure 1. The enhancement of yeast colony counts is also related to the degree of filter surface obscuration caused by spreading moulds (Figure 2). The lines fitted through the data points in the figures were calculated using the least squares method and were selected from among other curve types on the basis of the largest coefficient of determination.

5.0 DISCUSSION

The usefulness of agar media for the recovery of yeasts from aquatic environments using MF is dependent on the suppression of bacterial growth on the filter surface. Bacterial suppression can be achieved by either medium acidification (media #9, #10, #15), the inclusion of antibiotics in the agar medium (media #1, #2, #5, #6, #7, #8, #11) - or a combination of both methods (media #3, #4, #12, #13, #14). It was observed that bacterial growth was adequately suppressed by each of the media evaluated in the present study.

Mould overgrowth of the membrane filter surface can interfere with both the growth and enumeration of yeast colonies. Media incorporation of rose bengal and low temperature (10 - 17°C) incubation have been used to facilitate yeast colony development whilst retarding mould overgrowth. Two of the media evaluated contained rose bengal: medium #6 (0.2 g/L) and medium #13 (0.035 g/L). Medium #13 proved to be the more efficient of the two ($P < 0.01$) in suppressing mould overgrowth

with the lake and 30 mL pond water samples. This apparent contradiction may result from the higher nutrient content of medium #6 (Table 11) which, if correct, suggests that an elevated supply of readily assimilateable organic nutrients may counteract the mould inhibiting effect of rose bengal. This subject warrants further investigation, as does the usefulness of incorporating alternative mould suppressing agents in yeast enumeration media.

The disposition of the media to mould overgrowth appeared to vary with both the type and quantity of sample filtered. In the case of the lake water samples, the nutrient rich media #2 and #6 suppressed mould overgrowth poorly. Whereas, with the river water (medium #2), 5 mL pond water (media #11, #12, #10) and 30 mL pond water (media #11, #6, #5, #10, #14, #8 and #12) samples the media in parantheses, all of which are nutrient rich (>20 g/L; Table 11), were poor suppressors of mould overgrowth. Media #2 and #4, both of which are nutrient rich and efficient yeast recovery media, differed significantly from one another in their susceptibility to mould overgrowth when used to analyze the river water sample. The greater susceptibility of medium #2 to mould overgrowth might be explained by its combined peptone, yeast extract, and malt extract content which at 20 g is higher than the 11 g level of medium #4. Of the low nutrient media, only medium #1 suppressed mould overgrowth poorly, and then only with the Lake Ontario sample. Overall, the low nutrient medium #3 (Table 11), which was the only medium to contain neither peptone, yeast extract, nor malt extract, and was also the only medium to contain yeast nitrogen base and added ammonium sulfate, was consistently resistant to mould overgrowth. The apparent relationship between the nutrient content of yeast enumeration media and their susceptibility to mould overgrowth requires elucidation.

The rejection of yeast colony counts from membrane filters that had $\geq$ 25% of their surface area obscured by spreading moulds resulted in significantly higher yeast counts for the river, pond (30 mL samples), and lake water samples, which indicates that the elimination

of yeast counts from mould obscured filters can lead to higher estimates of aquatic yeast populations.

The performances of individual media were influenced by the type of water analyzed. Media #2 and #4 recovered more yeasts from the lake water than did the other media, with the exception of medium #1. Medium #4 recovered more yeasts from the river water than did the other media. Medium #2 recovered more yeasts from the 5 mL pond water samples than did the other media, with the exception of medium #13, a low nutrient medium that performed better than 50% of the media. Medium #13 and the low nutrient medium #3 also performed well with the 30 mL pond sample. Media #2 and #4 (Table 11) are nutrient rich media that contain glucose, peptone, yeast extract, and malt extract. Media #8 and #11 were the only other media to contain each of the forgoing ingredients. Medium #8 yielded high yeast counts with the 30 mL pond water samples. Medium #11, which is based on the commercially formulated Dicfo[1] agar, had lower levels of yeast extract and malt extract than media #2 or #4, and contained an additional ingredient, beef extract; these differences may have contributed to the dissimilarities observed between the performances of the former and latter media.

The media performance rankings for the lake and river water sample were correlated ($P = 0.01$), which indicates that for this pair of samples the type of water sample filtered did not influence the comparative abilities of the agar media to resuscitate yeasts. However, the media performance rankings differed for the pond/river and pond/lake sample type combinations. These initial observations suggest that the type of water sample analyzed can influence the relative performances of yeast recovery media.

In conclusion, there appears to be an inherent compromise associated with the design or selection of agar media for the recovery of viable yeasts from fresh water: media containing glucose that were

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[1]Difco is the trade mark of Difco Laboratories Inc.

also rich in peptone, yeast extract, and malt extract (e.g., medium #2) tended to recover yeasts efficiently but also tended to be susceptible to mould overgrowth; evidently, the incorporation of mould inhibiting agents in such media should receive more attention. Whereas low nutrient media, which appeared to be more resistant to mould overgrowth, tended to underestimate yeast populations. Although medium selection may ultimately depend on the nature of the mycolflora in the body of water under examination, if required, the following measures can help to limit the impact of spreading moulds on yeast population estimates: (1) selection of a low nutrient medium which would also, however, probably result in lower yeast counts, (2) increased sample replication and rejection of yeast counts obtained from mould obscured filters. If spreading moulds are not a problem, the use of a medium rich in peptone, yeast extract, and malt extract can help to maximize yeast colony counts.

Adaptation of the foregoing information for use in surveys of yeasts in environmental waters, should enable an accurate and realistic assessment of the relationships between yeasts and faecal contamination, or organic enrichment of aquatic environments to be made. Information pertaining to the distribution of yeasts in aquatic environments would usefully supplement data obtained for other water pollution parameters and would thus provide water quality managers with an enhanced means of rapidly and inexpensively monitoring water quality.

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REFERENCES

Ahearn, D.G. F.J. Roth, Jr., and S.P. Meyers. 1968. Ecology and Characterization of Yeasts from Aquatic REgions of south Florida. Mar. Biol. 1:291-308.

Buck, J.D. 1975. Distribution of Aquatic Yeasts - Effect of Incubation Temperature and Chloramphenicol Concentration on Isolation. Mycopathologia 56:73-79.

Cook, W.L. 1970. Effects of Pollution on the Seasonal Population of Yeasts in Lake Champlain. In Recent Trends in Yeast Research, D. Ahearn (Ed.), Georgia State, Atlanta. Spectrum, Monogr. Ser. Arts and Sci. 1:107-112.

Dutka, B.J., Ed. 1978. Methods for the Microbiological Analysis of Wates, Waste Waters, and Sediments. Inland Waters Directorate, Canada Centre for Inland Waters, Burlington, Ontario.

Fell, J.W. 1974. Distribution of Yeasts in the Water Masses of the Southern Oceans. In Effects of the Ocean Environment on Microbial Activities. R.R. Colwell and R.Y. Morita (Eds.). University Park Press, Baltimore, Md., pp. 510-523.

Hedrick, L.R., M. Soyugenc and L. Larsen. 1966. Yeasts in Sediment Core Samples from Lake Michigan. Proc. 9th Conf. Great Lakes Res., pp. 27-37. Publ. No. 15, Great Lakes Res., Div., Univ. Mich., Ann Arbor, Mich.

Hedrick, L.R., M. Soyugenc, P. Dupont and R. Ambrosini. 1964. Yeasts in Lake Michigan and Lake Erie. Proc. 7th Conf. Great Lakes Res., pp. 77-83. Publ. No. 11, Great Lakes Res. Div., Univ. Mich., Ann Arbor, Mich.

Meyers, S.P., D.G. Ahearn and W.L. Cook. 1970. Mycological Studies of Lake Champlain. Mycologia 62:504-515.

Roth, F.J., D.G. Ahearn, J.W. Fell, S.P. Meyers and S.A. Meyer. 1962. Ecology and Taxonomy of Yeasts Isolated from Various Marine Substrates. Limnol. Oceanogr. 7:178-185.

Schneider, J. 1978. Fungi. In Microbial Ecology of a Brackish Water Environment, G. Rheinheimer (Ed.). Springer-Verlag, New York, pp. 90-102.

Sherry, J.P. and A.A. Qureshi. 1981. Isolation and Enumeration of Fungi Using Membrane Filtration. In Membrane Filtration, Applications, Techniques and Problems. Ed. B.J. Dutka, pp. 189-218. Marcel Dekker Inc., New York.

Simard, R.W. and A.C. Blackwood. 1971. Yeasts from the St. Lawrence River. Can. J. Microbiol. 17:197-203.

Woollett, L.L. and L.R. Hedrick. 1970. Ecology of Yeasts in Polluted Waters. Antonie van Leeuwenhock, J. Microbiol. Serol. 36:427-435.

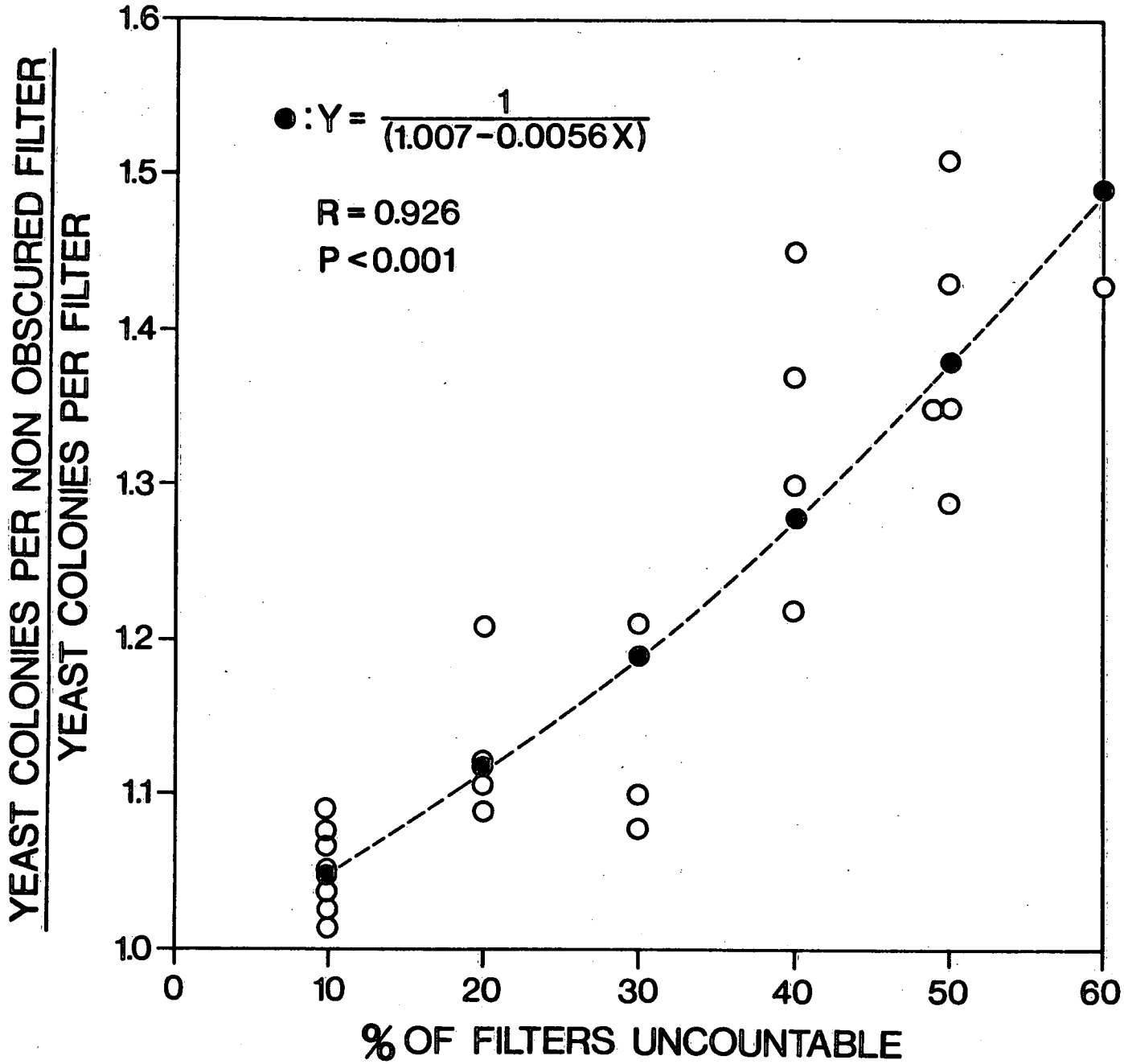
FIGURES

Figure 1. The relationship between the % of filters uncountable and the enhancement of yeast colony counts through the elimination of yeast counts from mould obscured filters.

R: correlation coefficient,
P: probability that R arises from chance.

Figure 2. The relationship between the degree of filter surface obscuration by spreading moulds and the enhancement of yeast colony counts through the elimination of yeast counts from mould obscured filters.

R: correlation coefficient,
P: probability that R arises from chance.


YEAST COLONIES PER NON OBSCURED FILTER
YEAST COLONIES PER FILTER
●: Y = 1 / (1.007 − 0.0056X)
R = 0.926
P < 0.001
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0
10
20
30
40
50
60
% OF FILTERS UNCOUNTABLE

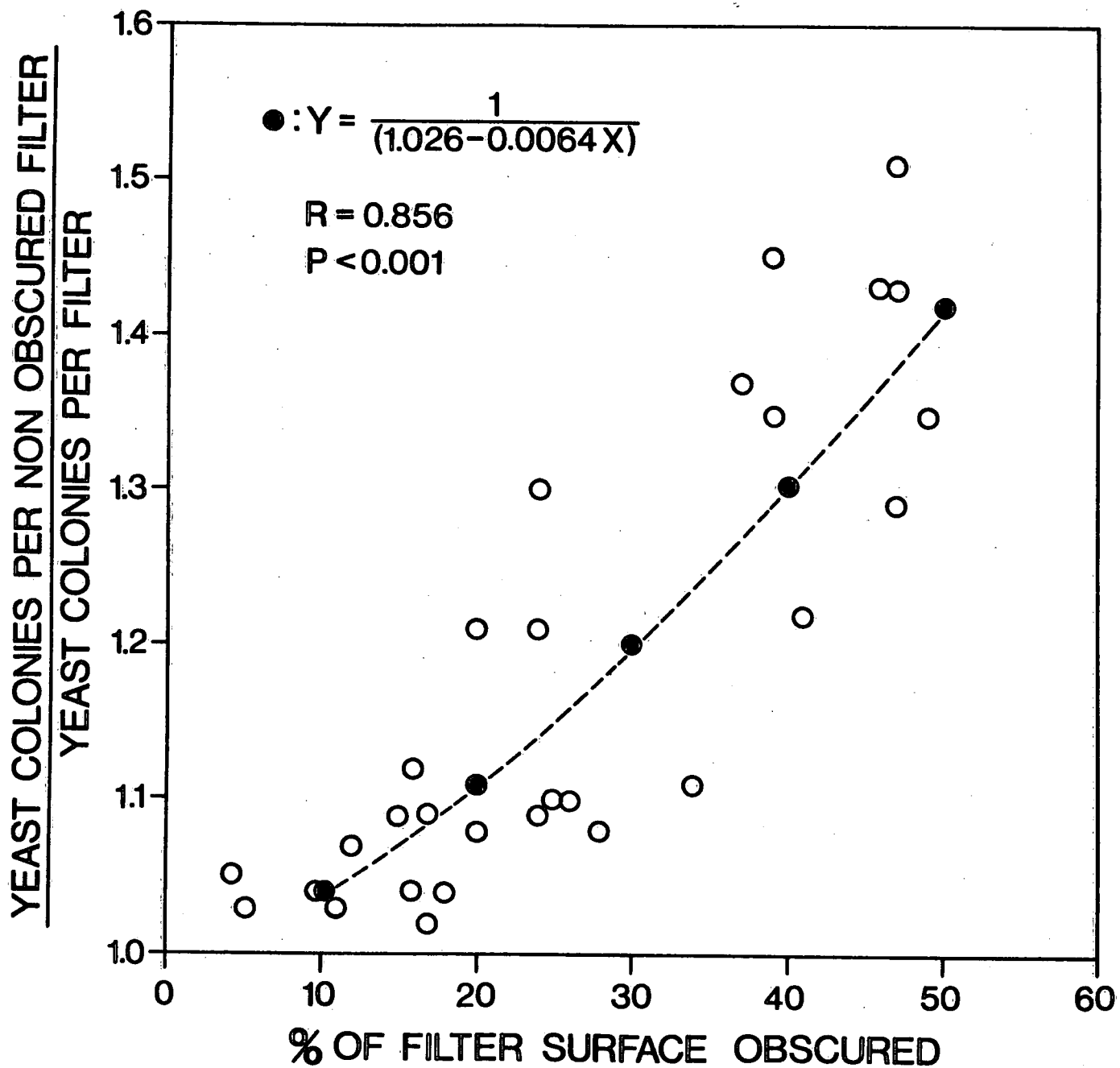

1.6
1.5
1.4
1.3
1.2
1.1
1.0
0
10
20
30
40
50
60
●: Y = 1 / (1.026 − 0.0064 X)
R = 0.856
P < 0.001
YEAST COLONIES PER NON OBSCURED FILTER / YEAST COLONIES PER FILTER
% OF FILTER SURFACE OBSCURED

TABLE 1. Media List

No.	Medium Name	Reference	Modifications
1	Distilled water Agar	Roth et al., 1962	Distilled H_2O substituted for sea water;*
2	YM Agar	Hedrick et al., 1964	
3	Yeast Isolation Medium	Hedrick et al., 1966	6.7 g/L yeast nitrogen base (Difco) substituted for basal salts and vitamins;*
4	Yeast Isolation Medium A	Ahearn et al., 1868	Lactic acid included;*
5	Yeast Isolation Medium B	Ahearn et al., 1968	
6	Yeast Isolation Medium C	Ahearn et al., 1968	*
7	Yeast Isolation Medium D	Ahearn et al., 1968	
8	YM Agar	Cook, 1970	
9	m-12 Agar	Meyers et al., 1970	*
10	Yeast Isolation Medium E	Fell, 1974	
11	Y Agar	Buck, 1975	Chloramphenicol concentration: 300 mg/L
12	Yeast Enumeration Agar	Schneider, 1978	Distilled H_2O substituted for aged seawater
13	mARGPA	Sherry & Qureshi, 1981	
14	mSTMEA	Sherry & Qureshi, 1981	
15	Yeast Extract Agar	Simar & Blackwood, 1971	

* Agar concentration : 15 g/L

TABLE 2. Ability of Media to Recover Viable Yeasts from Lake Ontario Water

No.	Yeast Colonies per Filter	Standard Deviation	Yeast Colonies per Nonobscured[1] Filter	Standard Deviation	% of Filters Uncountable[2]	% of Filter Surface Obscured by Spreading Moulds
1	31.3	10.1	34.1	5.0	10	24
2	29.5	17.1	40.5	7.2	40	36
3	31.2	6.5	31.2	6.5	0	5
4	39.9	5.7	39.9	5.7	0	10
5	29.9	4.9	31.1	3.2	10	10
6	25.0	8.6	26.9	6.6	10	20
7	29.6	4.7	30.7	3.5	10	18
8	30.7	5.9	30.7	5.9	0	12
9	23.6	7.3	25.7	3.5	10	17
10	32.9	4.0	32.9	4.0	0	10
11	32.0	5.6	32.0	5.6	0	12
12	28.2	4.6	28.2	4.6	0	8
13	31.4	5.1	32.2	4.6	10	9
14	31.4	4.5	31.4	4.5	0	11
15	25.1	5.2	25.0	5.2	0	11

[1] $<$ 25% of filter surface obscured by spreading moulds.
[2] $\geq$ 25% of filter surface obscured by spreading moulds.

TABLE 3. Probability that the media in column A[1] recovered significantly[2] more yeast colonies from the Lake Ontario water sample than did the row media. Medium performance is based on two enumeration procedures.

A	(a) Yeast Colonies per Filter						
4	10** 5***	11** 7***	13** 12***	14** 15***	1* 6***	3** 9***	8***
10	12*	15**	6*	9**			
11	15**	6*	9**				
13	15*	9*					
14	15**	9*					
3	15*	9*					
8	15*	9*					
5	15*	9*					
7	9*						

A	(b) Yeast Colonies per Non-Obscured Filter						
2	10* 8*	13* 12**	11* 6**	14** 9***	3* 15**	5**	7**
4	1* 7***	10** 8**	13** 12**	11** 6***	14** 9**	3** 15**	5***
1	12*	6*	9**	15**			
10	12*	6*	9**	15**			
11	9**	15**					
14	9**	15**					
3	9*	15*					
5	9**	15**					
7	9**	15*					
8	9*	15*					

[1] Media are arranged in order of decreasing colony counts.
[2] *P < 0.05; ** P < 0.01, *** P < 0.001. No significant difference detected between unlisted pairs of media.

TABLE 4. Ability of Media to Recover Viable Yeasts from Lake Ontario Water
(1 mL sample portions)

No.	Yeast Colonies per Filter	Standard Deviation	Yeast Colonies per Nonobscured[1] Filter	Standard Deviation	% of Filters Uncountable[2]	% of Filter Surface Obscured by Spreading Moulds
1	33.8	16.8	41.2	11.4	40	41
2	39.6	10.1	43.6	8.2	50	53
3	43.9	9.8	43.9	9.8	0	6
4	57.7	13.5	63.4	10.6	30	25
5	23.2	13.7	31.4	15.5	50	49
6	24.5	13.2	35.0	11.0	60	47
7	36.8	11.9	40.8	9.5	20	34
8	26.1	14.0	33.6	7.2	50	47
9	9.4	6.7	13.4	4.3	50	46
10	30.6	8.3	33.0	5.2	30	29
11	32.5	21.8	47.2	10.7	40	39
12	27.7	12.0	33.6	6.1	30	24
13	35.1	13.9	45.5	2.3	40	24
14	34.0	16.1	41.1	6.5	20	20
15	17.3	12.8	26.2	4.1	50	47

[1] $<$ 25% of filter surface obscured by spreading moulds.
[2] $\geq$ 25% of filter surface obscured by spreading moulds.

TABLE 5. Probability that the media in column A[1] recovered significantly[2] more yeast colonies from the Grand River samples than did the row media. Medium performance is based on two enumeration procedures.

A	(a) Yeast Colonies per Filter						
4	3*	2**	7**	13**	14**	1**	11**
	10***	12***	8***	6***	5***	15**	9**
3	10**	12**	8**	6**	5**	15***	9***
2	6*	5*	15***	9***			
7	6*	5*	15**	9***			
13	15*	9***					
14	15*	9**					
1	15*	9**					
11	9*						
10	15*	9**					
12	9**						
8	9**						
6	9**						
5	9*						

A	(b) Yeast Colonies per Non-Obscured Filter						
4	11*	13***	3**	2**	1**	14**	7***
	6***	12***	8***	10***	5***	15***	9***
11	12*	8*	10**	15***	9***		
13	6*	12***	8**	10***	15***	9***	
3	12*	10*	15**	9**			
2	12*	10*	15***	9***			
1	15*	9***					
14	12*	10*	15***	9***			
7	15**	9***					
6	9***						
12	15*	9***					
8	9***						
10	15*	9***					
5	9***						
15	9***						

[1] Media are arranged in order of decreasing colony counts.
[2] *P < 0.05; ** P < 0.01, *** P < 0.001. No significant difference detected between unlisted pairs of media.

TABLE 6. Ability of media to recover viable yeasts from pond water
(5 mL sample portions)

No.	Yeast Colonies per Filter	Standard Deviation	Yeast Colonies per Nonobscured[1] Filter	Standard Deviation	% of Filters Uncountable[2]	% of Filter Surface Obscured by Spreading Moulds
1	14.3	4.0	14.3	4.0	0	1
2	18.5	3.3	18.5	3.3	0	3
3	14.4	3.1	14.4	3.1	0	0
4	12.1	2.1	12.1	2.1	0	2
5	11.9	3.7	12.2	3.7	10	5
6	13.3	3.5	13.3	3.5	0	3
7	13.8	2.3	13.8	2.3	0	1
8	12.6	3.3	12.6	3.3	0	1
9	14.3	2.1	14.3	2.1	0	2
10	12.5	3.9	12.5	3.9	0	7
11	10.6	3.3	11.3	2.5	10	12
12	13.0	3.8	13.0	3.8	0	7
13	17.0	4.5	17.0	4.5	0	2
14	11.5	3.3	12.1	2.9	10	4
15	13.9	3.0	13.9	3.0	0	4

[1] $<$ 25% of filter surface obscured by spreading moulds.
[2] $\geq$ 25% of filter surface obscured by spreading moulds.

TABLE 7. Probability that the media in column A[1] recovered significantly[2] more yeast colonies from the pond water (5 mL sample portions) than did the row media. Medium performance is based on two enumeration procedures.

A	(a) Yeast Colonies per Filter						
2	3**	1*	9**	15**	7**	6**	12**
	8**	10**	4***	5***	14***	11**	
13	12*	8*	10*	4**	5*	14**	11**
3	11*						
1	11*						
9	4*	14*	11**				
15	11*						
7	11*						

A	(b) Yeast Colonies per Non-Obscured Filter						
2	3**	1*	9**	15**	7**	6**	12**
	8**	10**	5**	4***	14***	11**	
13	12*	8*	10*	5*	4**	14*	11**
3	11*						
9	4*	11*					
7	11*						

[1] Media are arranged in order of decreasing colony counts.
[2] *P < 0.05; ** P < 0.01, *** P < 0.001. No significant difference detected between unlisted pairs of media.

TABLE 8. Ability of media to recover viable yeasts from pond water
(30 mL sample portions)

No.	Yeast Colonies per Filter	Standard Deviation	Yeast Colonies per Nonobscured[1] Filter	Standard Deviation	% of Filters Uncountable[2]	% of Filter Surface Obscured by Spreading Moulds
1	*	-	*	-	(90*)	-
2	*	-	*	-	(90*)	-
3	80.2	10.4	80.2	10.4	0	1
4	*	0 *	-	(80*)	-	
5	63.0	6.4	64.4	4.8	10	17
6	59.2	10.9	64.9	7.1	30	26
7	67.1	6.9	67.1	6.9	0	2
8	76.2	18.7	83.0	10.2	20	15
9	65.4	9.7	65.4	9.7	0	2
10	68.4	13.5	71.1	11.1	10	16
11	43.6	19.5	59.0	12.9	50	39
12	67.4	6.9	67.4	6.9	0	8
13	82.9	4.0	82.9	4.0	0	4
14	53.9	15.5	60.1	8.0	20	16
15	78.8	8.0	78.8	8.0	0	7

* Yeast colonies difficult to enumerate because of colony merging.
[1] < 25% of filter surface obscured by spreading moulds.
[2] ≥ 25% of filter surface obscured by spreading moulds.

TABLE 9. Probability that the media in column A recovered significantly more yeast colonies from the pond water (30 mL sample portions) than did the row media. Medium performance is based on two enumeration procedures.

A	(a) Yeast Colonies per Filter			
13	10**	12***	7***	9***
	5***	6***	14***	11***
3	10*	12**	7**	9**
	5***	6***	14***	11***
15	10*	12**	7**	9**
	5***	6***	14**	11**
8	5*	6*	14**	11**
10	14**	11**		
12	14*	11**		
7	14*	11**		
9	14*	11**		
5	11**			
6	11*			

A	(b) Yeast Colonies per Non-Obscured Filter			
8	10**	12**	7**	9**
	6**	5***	14***	11**
13	10**	12**	7**	9**
	6***	5***	14***	11***
3	10**	12**	7**	9**
	6**	5***	14***	11**
15	10**	12**	7**	9**
	6**	5***	14**	11**
10	14*			

Media are arranged in order of decreasing colony counts.
*P < 0.05; ** P < 0.01, *** P < 0.001. No significant difference detected between unlisted pairs of media.

TABLE 10. Probability that spreading moulds from the water samples obscured a significantly greater filter surface area on the media in column A than on the Row media.

A	a) Lake Ontario	A	c) Pond Water (5 mL)
14	3*	6	3*
11	3*	15	3*
6	3** 12** 13** 4* 10* 15* 14* 15*		3* 1*
1	3* 12*	10	3** 1* 8* 7*
2	3* 12*	12	3*** 1*** 8*** 7*** 13** 4** 9** 6** 2* 15* 14** 5*
		11	3*** 1** 8* 7* 13* 4* 9*

A	b) Grand River	A	d) Pond Water (30 mL)
7	3** 14*	13	3*
11	3*	15	3*
1	3*	12	3** 9* 7*
9	3*	8	3** 9* 7**
8	3*	14	3*** 9** 7**
6	3** 14*	10	3*** 9*** 7*** 13** 15*
5	3**	5	3*** 9*** 7*** 13*** 15**
2	3*** 14* 13* 12* 4*	6	3*** 9*** 7** 13** 15*
		11	3*** 9** 7*** 13** 15**

*P < 0.05; ** P < 0.01, *** P < 0.001. No significant difference detected between unlisted pairs of media. Media are arranged in order of increasing surface observation.

TABLE 11. Nutrient Content of the Test Media
Ingredient (g/L)

Medium	Glucose	Peptone	Yeast Extract	Malt Extract	Yeast Nitrogen Base	Beef Extract	Corn Meal	Meat Extract	Total
1	10	5	1	0	0	0	0	0	16
2	10	10	5	5	0	0	0	0	30
3	10	0	0	0	6.7	0	0	0	16.7
4	10	5	3	3	0	0	0	0	21
5	10	9	1	0	0	3	0	0	23
6	20	10	1	0	0	0	0	0	31
7	0	0	0.1	0.1	0	0	6.2	0	6.4
8	10	10	3	3	0	0	0	0	26
9	0	5	3	20	0	0	0	0	28
10	20	5	1	0	0	3	0	0	29
11	20	5	1	2	0	3	0	0	31
12	20	5	1	0	0	0	0	2.4	28.4
13	10	5	0	0	0	0	0	0	15
14	0	5	0	30	0	0	0	0	35
15	20	10	5	0	0	0	0	0	35

APPENDIX 1

APPENDIX: Formulae for Media

Medium #1. Seawater agar (Roth et al., 1962)

Glucose	10.0 g
Peptone	5.0 g
Yeast extract	1.0 g
Agar	*
Aureomycin	100.0 mg**
Chloramphenicol	120.0 mg**
Streptomycin sulfate	20.0 mg**
Seawater	1000 mL

Medium #2. YM agar (Hedrick et al., 1964)

Glucose	10.0 g
Yeast extract	5.0 g
Malt extract	5.0 g
Peptone	10.0 g
Agar	15.0 g
Streptomycin	100.0 mg
Penicillin	100.0 mg
Chloromycetin	50.0 mg
Distilled water	1000 mL

Medium #3. Yeast isolation medium (Hedrick et al., 1966)

Basal salts and vitamins (Wickerham, 1946)***

Glucose	10.0 g
Ammonium sulfate	5.0 g
Agar	*
Streptomycin	100.0 mg
Penicillin	10.0 EX5 units
Chloromycetin	50.0 mg
Lactic acid (85%)	1.5 mg
Distilled water	1000 mL

Prior to the addition of the agar, the pH of the medium was adjusted to 4.0-4.5.

Medium #4. Yeast isolation medium A (Ahearn et al., 1968)

Dextrose	10.0 g
Malt extract	3.0 g
Peptone	5.0 g
Yeast extract	3.0 g
Agar	*
Chloramphenicol	0.5 g
Lactic acid	10.0 mL
Distilled water	1000 mL

Medium #5. Yeast isolation medium B (Ahearn et al., 1968)

Dextrose	10.0 g
Yeast extract	1.0 g
Nutrient agar	23.0 g
Peptone	4.0 g
Chloramphenicol	0.5 g
Distilled water	1000 mL

Medium #6. Yeast isolation medium C (Ahearn et al., 1968)

Dextrose	20.0 g
Neopeptone	10.0 g
Yeast extract	1.0 g
Agar	*
Rose bengal	0.2 g
Chloramphenicol	0.5 g
Distilled water	1000 mL

Medium #7. Yeast isolation medium D (Ahearn et al., 1968)

Malt extract	0.1 g
Yeast extract	0.1 g
Cornmeal agar	8.0 g
Agar	5.0 g
Chloramphenicol	0.5 g
Distilled water	1000 mL

Medium #8 YM agar (Cook, 1970)

Glucose	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Malt extract	3.0 g
Agar	20.0 g
Declomycin	150.0 mg
Penicillin	100.0 mg
Chloramphenicol	100.0 mg
Distilled water	1000 mL

Medium #9 m-12 agar (Meyers et al., 1970)

Dimalt-20	20.0 g
Bactopeptone	5.0 g
Bacto-yeast extract	3.0 g
Agar	*
Distilled water	1000 mL

Following sterilization, the cooled medium was acidified to pH 4.3-4.5 with sterile lactic acid (10%) to retard bacterial growth.

Medium #10 Yeast isolation medium (Fell, 1974)

Difco nutrient agar	23.0 g
Agar	2.0 g
Glucose	20.0 g
Yeast extract	1.0 g
Water	1000 mL

pH adjusted to 4.5 with HCl.

Medium #11 Y agar (Buck, 1975)

Glucose	20.0 g
Difco nutrient agar, pH 6	23.0 g
Malt extract	2.0 g
Difco yeast extract	1.0 g
Chloramphenicol	0.3 g
Distilled water	1000 mL

Medium #12 Yeast enumeration agar (Schneider, 1978)

Difco peptone	5.Ø g
Meat extract	2.4 g
Glucose	2Ø.Ø g
Yeast extract	1.Ø g
$FePO_4 . 4H_2O$	Ø.Ø1 g
Agar	15.Ø g
Distilled water	1ØØØ mL
Streptomycin sulfate	Ø.25 g
Binotal	Ø.25 g

After sterilization, the pH was adjusted with lactic acid (1Ø%) to 4.Ø-4.5 aqnd the antibiotics were then added to the solution.

Medium #13 Modified aureomycin-rose bengal-glucose-peptone agar (mARGPA) (Dutka, 1978)

Glucose	1Ø.Ø g
Peptone	5.Ø g
KH_2PO_4	1.Ø g
$MgSO_4 . 7H_2O$	Ø.5 g
Agar	2Ø.Ø g
Distilled water	1ØØØ mL
Rose bengal	Ø.Ø35 g
Aureomycin HCl	2ØØ.Ø mg

The pH was adjusted to 5.4, and the medium was sterilized at 121 C for 15 min. Membrane filter (Ø.2um) sterilized aureomycin was added before pouring plates.

Medium #14 Modified streptomycin-terramycin-malt extract agar (MSTMEA) (Dutka, 1978)

Malt extract	3Ø.Ø g
Peptone	5.Ø g
Agar	15.Ø g
Distilled water	1ØØØ mL
Streptomycin	2ØØ.Ø mg
Terramycin	2ØØ.Ø mg

The pH was adjusted to 5.4, and the medium was sterilized at 121 C for 15 min. Membrane filter (Ø.2 um) sterilized streptomycin and terramycin were added before pouring plates.

Medium #15 Yeast extract agar (Simard and Blackwood, 1971)

Yeast extract	5.Ø g
Peptone	1Ø.Ø g
Glucose	2Ø.Ø g
Agar	2Ø.Ø g
Distilled water	1ØØØ mL

After sterilization, the medium was acidified with sterile 1Ø% lactic acid to a final pH of 4.5.

* Agar concentration not specified, used 15 g/L.
** Originally expressed as mg%.
*** Quantity not specified, used 6.7 g/L yeast nitrogen base (Difco).